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MINIREVIEWS

Hepatitis B and pregnancy: An update review article

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Abstract

Chronic hepatitis B, as a global health problem, is a disease that begins in the prenatal period and its complications gradually become clear later in life. About 5% of women worldwide are carriers of chronic hepatitis B virus (HBV). The most common method of transmission of HBV around the world is from mother to infant. This article aims to review the unique challenges of hepatitis B in pregnancy. Data for this review were collected from our previous studies and experiences plus various data banks, such as PubMed, EMBASE, ISI Web of science, Scopus, Google Scholar and Iranian databases. A comprehensive search was performed using the combinations of the keywords to review relevant literature and higher education journals. All published data up to February 2014 have been included in this review. This article addresses several interesting aspects. First, hepatitis B in pregnancy can vary regarding prevalence, virus behavior, prenatal transmission and outcome of the pregnancy. Second, the women of reproductive age with chronic HBV remain a major source for continued spread of the virus. Finally, pregnant women need screening in prenatal care to enable early intervention when necessary.

Key words: Hepatitis B; Pregnancy; Screening; Prenatal transmission; Treatment

Core tip: Chronic hepatitis B is a global health problem. About 5% of women worldwide are carriers of chronic hepatitis B virus (HBV). The most common method of transmission of HBV around the world is from mother to infant. We are gathering an increasing amount of interesting aspects to accurately describe the unique challenges of hepatitis B in pregnancy. This article addresses hepatitis B in pregnancy which can vary regarding prevalence, virus behavior, prenatal transmission, screening and outcome of the pregnancy.

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INTRODUCTION

Hepatitis B is a global health problem[1] and the leading cause

of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma[2]. The World Health Organization has announced that Hepatitis B, after smoking tobacco, is the second most important human carcinogen[3]. Hepatitis B is 50 to 100 times more contagious than the human immunodeficiency virus (HIV)[4] and Americans are suffering from it four times more than HIV. Given that one out of every 15 people worldwide has hepatitis B, it has become the most common blood borne infectious disease in the world[5]. According to estimates, around 2 billion people worldwide are infected by the hepatitis B virus (HBV) and about 360 million live with chronic infection, while at least 600 thousand people lose their lives per year due to acute or chronic consequences of hepatitis B[4]. Iran is located in the Middle East region and according to the centers for disease control and prevention, it has an average prevalence of chronic HBV[6]. Chronic hepatitis B is a disease that begins in the perinatal period and its complications gradually become clear later in life[7]. About 5% of women worldwide are carriers of chronic HBV[8]. The most common method of transmission of HBV around the world is from mother to infant and this infection might occur during the intrauterine or perinatal period[1]. HBV prevalence in pregnant women is approximately 5% and this varies from 0.6% in low prevalence areas to over 20% in areas with a high incidence in the Far East and Africa[9]. This article aims to review the unique challenges of hepatitis B in pregnancy.

SEARCH STRATEGY

Data for this review were collected from our previous studies and experiences plus various data banks, such as PubMed, EMBASE, ISI Web of science, Scopus, Google Scholar and Iranian databases including Iranmedex and SID. A comprehensive search was performed using the combinations of the keywords "Hepatitis B, pregnancy, prenatal transmission, vaccination, treatment" to review relevant literature and higher education journals. The searches were done by using Boolean operators OR, AND between main phrase and the mentioned keywords were extracted from specific themes of the topic under study. A search strategy was built, applying the advanced search capability of the search engine. The inclusion criteria as set out was that only articles that explicitly dealt with Hepatitis B in pregnancy were included. We also looked at the reference list of the retrieved papers and searched other search engines. A total of 150 articles were found in the primary search but after elimination of duplicates or irrelevant papers, only 60 records were reviewed. All published data from 1999 to 2014 have been included in this review.

RESULT

Natural history of HBV

HBV can exist in many body fluids such as blood, saliva, semen and vaginal fluid. The virus can survive outside the body for more than 7 d. So, if scratched skin is in contact with an infected surface, infection occurs. When hepatitis B infection occurs, the patient enters the incubation period which is asymptomatic and the patient is usually unaware of the infection. During this period, liver transaminases are normal. Although the factors that affect the length of the incubation period are perhaps unknown, probably factors such as the size of HBV, the binding ability of cell surface receptors to the virus and the host immune system are involved. The incubation period may be as long as 2 to 6 mo with an average of 60 d[10]. The clinical appearance of hepatitis B infection varies depending on the age and the host immune system. Children under 5 years and adults with weakened immune systems are asymptomatic. Only 30% of people with acute infection will develop jaundice. Jaundice may be mild or severe, depending on the host's immune system. The final phase of chronic HBV infection is recovery, in which transaminases are decreased and the clinical signs are lowered. Viral hepatitis is an inflammatory widespread phenomenon which can cause acute or chronic liver damage. The main mechanisms of hepatocyte injury are unclear although specific and nonspecific antigens are involved in hepatocyte injury. Evidence suggests that the characteristics and clinical outcomes after acute liver injury are associated with viral hepatitis and are determined by immunological responses of the host. Outcome of HBV infection varies from full recovery to progression to chronic hepatitis and death from fulminant hepatitis is rarely seen[11-13].

Natural history of HBV in pregnancy

In areas of high prevalence, most patients with chronic HBV are women of reproductive age[14]. Transmission from mother to fetus during prenatal or horizontal transmission in childhood are the main ways of transmission of HBV in areas with a high and moderate prevalence. Also, in areas with low prevalence, unsafe sexual activity is the most common way of transmission[15]. Therefore, women of reproductive age are considered an important source of infection[16]. Risk factors for chronic HBV carriers in the reproductive age population are unknown. The factors associated with chronic carrier status include resident status, positive family history, no history of previous vaccination and previous hepatitis B surface antigen (HBsAg) testing[17]. Chronic HBV infection is likely to be a significant cause of infertility. HBV infection reduces fertilization ability during *in vitro* fertilization and embryo transfer[18]. The clinical course of HBV infection does not change during pregnancy and usually there is no difference in pregnant and nonpregnant women. It has been identified that hepatitis flares occur rarely during pregnancy, while its indices increase after delivery. There are controversies regarding the disease complications in pregnant women and fetuses. Some believe that chronic carriers of HBV in pregnancy are associated with increased rates of miscarriage, gestational diabetes and preterm labor[19]. The assessment of fetal distress in pregnant women with HBV showed that HBV infection can cause chorion angiopathy and decrease placental function, leading to fetal distress[20]. Available information indicates that HBV transmission

via the placenta is not as common as previously thought; actually, viral DNA is rarely found in amniotic fluid or cord blood[5]. Most infant infection in the womb is caused by the mother's blood transfusion to the fetus during uterine contractions or rupture of fetal membranes[8] or by vertical transmission perinatally by exposure to blood or secretions of an infected birth canal of the mother[21]. It is estimated that 50% of cases of chronic hepatitis B are results of vertical transmission or acquired in early childhood[8]. The mechanisms through which HBV infections are transmitted in the uterus are controversial and being reviewed. Some of these assumptions include transmission through the placenta, transfer through placental leakage, cracks in the placental barrier, mononuclear cells in peripheral blood and transmission through the father[22]. Intrauterine transmission of HBV infection occurs *via* two pathways: (1) blood release (hematogenous) that causes the infection of placental vascular endothelial cells and is probably the main route for infection transmission; and (2) cellular transport through cell by cell. One of the explained mechanisms of the intracellular transport route is binding of HBsAg-anti-HBsAb with Fc-γ receptor Ⅲ[10].

A study of 402 infants of HBsAg-positive mothers showed that the risk of intrauterine transmission depended on HBeAg-positive mothers and virus existence in vascular endothelial cells in chorionic villi[23]. The most important risk factors for mother-to-child transmission (MTCT) include: HBV DNA > 200000 IU (106 copies)/mL, HBeAg positive and pregnancy complications such as preterm delivery, prolonged labor and prior failure of immunoprophylaxis in sibling(s). Antiviral therapy in the last stages of pregnancy is the most effective way to reduce transmission of infection from the mother. Besides, elective cesarean delivery can reduce the risk of transmission[24]. The prevalence of HBV infection in infants and children are different depending on race and ethnicity, with Asian women having the highest prevalence (6%)[25].

MTCT

Hepatitis B virus can be integrated in the placenta, leading to the infection. In Wang's study of 15 HBeAg-positive mothers and their babies, he revealed that HBeAg actually crosses the human placenta. In the case of simultaneous TORCH (Toxoplasmosis, Other Agents, Rubella, Cytomegalovirus, Herpes Simplex) infections that can cause placental cracks or a damaged placental barrier, neonatal HBV infection further increases. Also, HIV infection increases the risk of transmission of HBV infection[26]. In another study, Elefsiniotis *et al*[27] examined serological and virological profiles of cord blood samples taken from mothers with negative HBeAg and revealed that in almost a third of these mothers, HBsAg, despite maternal viral load, HBV pathology and type of delivery, can pass through placental barrier. Chronic HBV in pregnant women is usually mild but the disease may flare up shortly after birth[28]. HbsAg-positive mothers that are also HbeAg-positive are more likely to transmit the disease to their infants (70% to 90%), while the HbsAg-positive and HbeAg-negative mothers have a lower infection transmission rate[21]. The risk of transmission in HbeAg-negative mothers is 10% to 40% and in affected infants, 40% to 70% of the infection becomes chronic[25].

Although infection in HbeAg-negative mothers occurs less commonly, infants in early periods of infancy often progress to acute or fulminant hepatitis. So, despite the HBeAg/Ab status of the mothers, prevention in infants is necessary[29]. The transmission rate in pregnant women with a positive serum viral DNA is 90%, while for pregnant women with negative viral DNA it was reported to be 10% to 30%[30,31]. Hepatitis B vaccine is safe for pregnant women in each trimester and those who are seronegative can be vaccinated during pregnancy. Serum level protection in pregnant women is only 45%. This is lower than that mentioned for nonpregnant women as well as for women during the postpartum period that had received three doses of the vaccine[32]. Vaccination during pregnancy, in addition to being beneficial for the mother, also provides partial immunity for the infant[33]. Studies showed that a maternal prenatal screening program and active and passive immunization of infants after delivery significantly decreased HBV infection to 95%[34]. Most infections of pregnant women are chronic and asymptomatic and are diagnosed in prenatal screening. These women are considered to have chronic hepatitis but antiviral therapy is generally not performed during pregnancy[3]. Perinatal HBV transmission accounts for about 21% of HBV-related deaths worldwide and 13%-26% regionally[35]. HBsAg was found to be positive in 50% of cord blood and 95% of gastric fluid samples of infants of HbsAg-positive mothers. During labor, the infant is in contact with the mother's blood which contains the virus and it is possible for him to swallow contaminated fluids, enabling neonatal infection by physiological transfusion or blood contact and the mother's birth canal secretions[10,36].

Global hepatitis B disease burden and impact of vaccination

Safe and effective vaccines have been available to prevent HBV infection since 1981 and the cost-effectiveness of hepatitis B vaccination has been well documented[37]. A study performed in Iran by Alavian[38] showed a significant reduction in the rate of HBsAg positivity in the subgroup of children aged 2-14 years after expanding the immunization program. HB vaccine can be fully justified on economic grounds in that either the cost-benefit ratio is positive or the cost-effectiveness ratio suggests the vaccine to be a good "buy" for the public health services or both[38]. Several studies have implicated high maternal viremia as the most important factor associated with failure of neonatal vaccination[39]. The key strategy to decrease mortality from HBV is to prevent infants from acquiring HBV infection. The Advisory Committee on Immunization Practices (ACIP) recommends that all newborns receive their first HB vaccination before hospital discharge[2]. For infants born to HBV-infected women, administering ACIP-recommended post-exposure prophylaxis of hepatitis B immune globulin (HBIG) and

HB within hours of birth followed by completion of the HB series has been shown to be 85%-95% effective in preventing HBV infection[40] .

From a few reported trials, HB vaccine and HBIG seem safe. Furthermore, cohort studies found that HB vaccination is well tolerated and that severe adverse events are rare[21]. One systematic review showed that HB vaccine, HBIG and vaccine plus immunoglobulin prevent hepatitis B occurrence in infants of mothers positive for HBsAg[21]. In a follow up study of 184 infants born to HBsAg carrier mothers, Wang *et al*[41] found that after infants were immunized by HBIG combining HB vaccine, the anti-HBs-positive rate reached 92% at 7 mo and gradually decreased thereafter. 72.04% of the infants at 24 mo and 60% at 36 mo showed detectable levels of anti-HBs[41]. Cost effectiveness studies showed that in countries with low, intermediate and high HBV prevalence, vaccination of infants of HbsAg-positive mothers had numerous advantages[21]. Infants who are infected with hepatitis B are generally asymptomatic but 90% of them develop chronic infection[42]. Coadministration of specific immunoglobulin and HB vaccine is highly effective in preventing infection. However, approximately 10% to 20% of infants are still chronically infected despite receiving this treatment in their early life. Intrauterine infection is the leading cause of HB vaccine failure in infants born to mothers with HBV[34].

Without immunoprophylaxis, up to 90% of infants born to HBeAg-positive mothers become HBV carriers. In comparison, 20% to 30% of children infected between the age of 1 to 5 years and fewer than 5% of immunocompetent adults become HBV carriers[43]. Intrauterine HBV infection has been suggested to be caused by transplacental transmission that cannot be blocked by the HB vaccine[23]. In a case-control study of 402 HBs Ag-positive pregnant women in China, Xu *et al*[23] found that 3.7% of their newborn were HBs Ag positive within 24 h of birth. The main risk factors for intrauterine HBV infection were maternal serum HBeAg positivity, history of threatened preterm labor and HBV in villous capillary endothelial cells in the placenta.

Joint immunoprophylaxis with HBIG and three doses of HB vaccines to infants born to HBsAg-positive mothers are known to be safe and effective. However, 5%-10% of infants of HBV-positive mothers become infected even with proper vaccination[21]. There are some cases of vaccination failure. Very high maternal viremia, intrauterine infection or escape mutants after the implementation of the universal vaccination program are possible reasons for vaccination failure. Immunocompromised hosts also risk vaccination failure[7]. Hence, health care providers must consider the maternal HBV DNA level in decision making regarding management options during pregnancy[25].

Treatment of HBV during pregnancy

HBV treatment in pregnancy is complicated[43]. For women with HBV who have intentions of becoming pregnant, treatment can be postponed in cases of mild disease until after delivery. In cases of moderate or severe disease or getting pregnant during treatment, it is necessary that the potential risks of treatment with antiviral drugs be evaluated against the risk of disease progression in the case of no treatment[19]. Pregnant women with low viral loads do not need immediate treatment, while for severely viral mothers (> 10^9 copies/mL), antiretroviral therapy in the last trimester of pregnancy should be considered[44]. Management of HBV treatment in pregnancy is unique. Aspects of care and treatment that need to be taken into consideration include: HBV effects on pregnancy; pregnancy effects on HBV and its complications; HBV treatment during pregnancy; and prevention of perinatal HBV infection[28]. Zhang *et al*[45] assessed the effects of telbivudine on HBV intrauterine infection during the last phases of pregnancy in 61 pregnant women and found that serum HBV DNA levels in patients treated with telbivudine were significantly lower than the control group ($P < 0.01$). Infection rate was reported to be 0% in infants receiving telbivudine and 13.3% in the control group. These researchers stated that treatment of HBV-infected mothers with telbivudine can block intrauterine infection[45].

Another study evaluating the effectiveness and safety of using HBIG during pregnancy to prevent mother to child transmission showed that infants of mothers receiving HBIG were less infected by intrauterine infection (indicated by HBsAg as OR = 0.22; indicated by HBV DNA as OR = 0.15; $P < 0.01$ for both) and a high level of safety and protection was observed in them (indicated by HBs Ab as OR = 11.79; $P < 0.01$)[46]. A randomized trial on interrupted HBV intrauterine transmission estimated that repeated HBIG injections to pregnant women before the delivery may block the HBV intrauterine infection by reducing the level of viremia[34]. Numerous studies reported that treatment with lamivudine in highly viral pregnant women prevents HBV replication. In the recent study, lamivudine (100 mg/d) therapy was administered to pregnant patients with HBV who had levels of HBV DNA greater than 10000 copies/mL after the 32nd gestational week. A 102 decrease in HBV viral load was observed in 71% of the patients[47].

Safety of medications used during pregnancy and lactation, the effect of the chosen drug, resistance to treatment, side effects and duration of the treatment are factors that should be considered in the treatment of pregnant women with hepatitis B. If delivery takes place in the near future, it is wise to delay treatment until after the delivery[48,49]. In women at high risk or with known HBV infection who want to be pregnant, it is necessary to determine the exact status of the disease so that an appropriate treatment plan can be scheduled. A therapeutic approach to hepatitis B during pregnancy involves three basic strategies as follows: (1) screening pregnant women and performing a routine HBsAg test at the first prenatal visit; (2) treatment of HBV infection in pregnant women; and (3) prevention of mother-infant transmission[3,25] (Figure 1).

Counseling and prenatal care in hepatitis B

Since health status during pregnancy depends on the

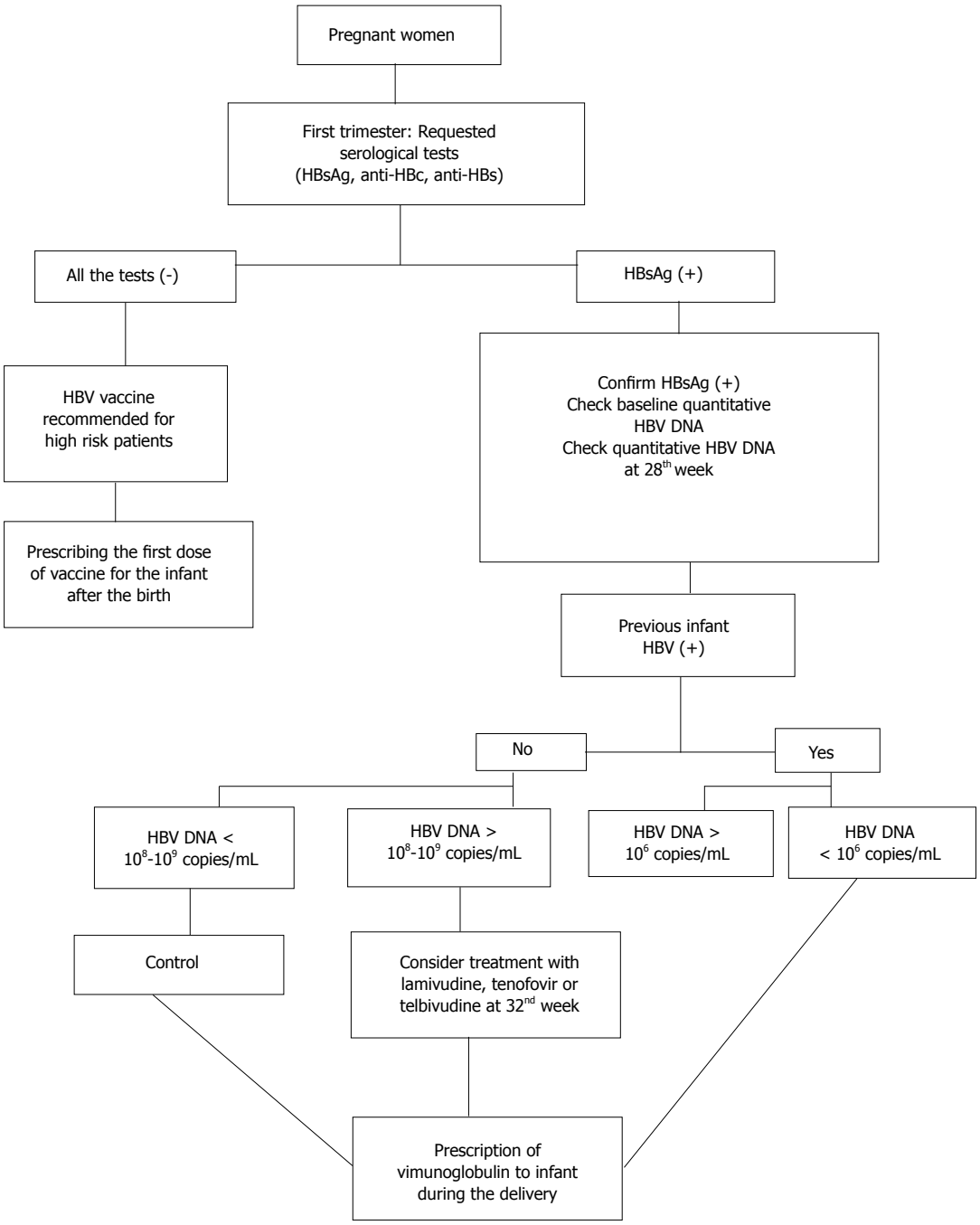

Figure 1 Hepatitis B virus treatment algorithm during pregnancy[43,44,50]. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; anti-HBc: Hepatitis B core antigen.

health status before it, pre-pregnancy counseling and care of patients with hepatitis should be an essential part of the patient's training. A comprehensive plan of care before pregnancy can help in reducing the risks, promoting healthy life styles and increasing preparation for pregnancy. Principles of pre-pregnancy counseling should include the following[51]: (1) high risk women should be screened and can be vaccinated against hepatitis B[52]; (2) the test for hepatitis B has been confirmed to be negative before starting the anonymous donor oocyte *in vitro* fertilization sequence[53]; (3) infected women should be counseled regarding the risks of the disease and transmission; (4) patients with hepatitis B should be referred for treatment before becoming pregnant; (5) before starting treatment, request a β-hCG test for the patient to ensure she is not pregnant since some drugs used to treat hepatitis are teratogenic for the fetus; (6) explain about effective contraceptives to suit each individual ; and (7) remind women not to take oral contraception, because their estrogenic effects on the liver are unknown[32].

It is important to consider a comprehensive health care program that includes an integrated approach to medical and psychosocial care for pregnant women with hepatitis B. Accurate and detailed information should be

obtained from the pregnant women regarding the history of previous delivery, using alcohol and drugs during pregnancy, serological hepatitis B test and immune status of the pregnant women. Monitoring the health status of the fetus and determining the next prenatal visits based on the patient's condition are among the other prenatal care needed. Although nutrition knowledge attempts to identify the ideal amount of nutrition groups for pregnant women, people who are directly responsible for the care of mothers with hepatitis B can perform their duties in the best way[54-56].

Breast feeding and hepatitis B

Breastfeeding is the foundation of infant nutrition and sets the scene for lifetime health. The World Health Organization recommends that all mothers who are hepatitis B positive breastfeed their infants and that their infants be immunized at birth[57]. A recent meta-analysis review reported breastfeeding after proper immunoprophylaxis did not contribute to mother-to-child transmission of HBV[58]. Mothers with hepatitis B are advised to pay attention and check the nipples before each feed and in case of any cracks, bleeding or any kind of blood on the nipples, to temporarily stop breastfeeding and express all the milk and discard it. When the nipples are healthy without any cracks, the milk should be expressed, kept in good condition and given to the infant at the time of cessation of breastfeeding. The mother should learn the correct way of breastfeeding and embracing the infant so that an uncomfortable position does not cause the infant to refuse breastfeeding. During teething, caution should be taken so that the nipple is not wounded and a milk bottle can be used at this time. To prevent mother to child transmission, the following actions are recommended: educate the mothers; isolate HbsAg-positive mothers during labor; clean blood and secretions from the infant's body; and active and passive immunization of infants soon after birth[59,60].

CONCLUSION

This article discusses the unique challenges of hepatitis in pregnancy and addresses several interesting aspects. First, hepatitis B in pregnancy can vary regarding prevalence, virus behavior, prenatal transmission and outcome of the pregnancy. Second, the women of reproductive age with chronic HBV infection remain a major source for continued spread of the virus. Finally, pregnant women need screening tests directed at virus detection in prenatal care to enable early intervention when necessary. In addition, we would like to control perinatal transmission and reduce the numbers of new carriers as much as possible with immune prophylaxis after delivery.

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ORIGINAL ARTICLE

Case control study

High prevalence of post-partum depression in women with coeliac disease

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Abstract

AIM: To explore the prevalence of post-partum depression (PPD) in coeliac disease (CD).

METHODS: We performed a case-control study evaluating the prevalence of PPD in CD patients on gluten-free diet (GFD) compared to that of healthy subjects experiencing a recent delivery. All participants were interviewed about menstrual features, modality and outcome of delivery and were evaluated for PPD by Edinburgh Postnatal Depression Scale (EPDS).

RESULTS: The study included 70 CD patients on GFD (group A) and 70 controls (group B). PPD was present in 47.1% of CD women and in 14.3% of controls ($P < 0.01$; OR = 3.3). Mean EPDS score was higher in CD compared to the controls (mean score: group A 9.9 ± 5.9; group B 6.7 ± 3.7; $P < 0.01$). A significant association was observed between PPD and menstrual disorders in CD (69.7% *vs* 18.9%; $P < 0.001$; OR = 3.6).

CONCLUSION: PPD is frequent in CD women on GFD, particularly in those with previous menstrual disorders. We suggest screening for PPD in CD for early detection and treatment of this condition.

Key words: Coeliac disease; Depression; Post-partum depression; Menstrual disorders; Gluten-free diet

Core tip: Some studies have shown an increased prevalence of psychological symptoms and mental disorders in patients affected by coeliac disease (CD) and depression appears to be the most important condition in undiagnosed CD. On the other hands, focused data on post-partum depression are still lacking. In our mind, the present work is the first study mainly focused on this interesting and relevant topic.

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INTRODUCTION

Coeliac disease (CD) is a chronic small intestinal immune-mediated enteropathy precipitated by the exposure to dietary gluten in genetically predisposed individuals[1,2]. CD is the most common cause of enteropathy in the western world and affecting around 1% of the general population in both children and adults[3].

Gluten consumption in these susceptible individuals leads to small bowel damage and the activation of immune responses which cause both intestinal and extra-intestinal manifestations of the disease.

About clinical features, as reported in a recent article by Ludvigsson *et al*[1], classical CD is characterized by signs and symptoms of malabsorption (diarrhoea, steatorrhoea, weight loss, *etc.*), while non-classical CD presents with anaemia, osteopenia/osteoporosis, recurrent abortions, hepatic steatosis, dental enamel hypoplasia, hypertransaminasemia, recurrent aphthous stomatitis.

At present, CD diagnosis requires first of all a serological screening of patients with suspected CD using anti-tissue transglutaminase (a-tTG) and anti-endomysial antibodies and then, a duodenal biopsy to assess the intestinal damage in patients with positive serology[1].

Many studies have shown an increased prevalence of psychological symptoms and mental disorders in patients affected by CD and depression appears to be the most important condition in undiagnosed CD[4-7]. In effect, Addolorato *et al*[8] reported that the prevalence of depression in CD patients was significantly higher than in the control group (57.1% *vs* 9.6%). In addition, recent reports have revealed a high prevalence of anxiety and sleep disorders in coeliac patients, so confirming the importance of exploring and treating mental aspects in this particular population[8,9].

Post-partum depression (PPD) affects 10%-15% of new mothers being the most common complication of pregnancy in developed countries[10,11]. This condition is often unrecognized and when left untreated can be associated with potentially adverse consequences for the mother, her infant, and her family. In particular, PPD can lead to disruptions in maternal-infant interactions, and lower cognitive functioning and behavioural problems in children[12,13]. Even if PPD is underdiagnosed, a number of important risk factors (past depression, stressful life events, poor marital relationship, and social support) have been described and can be utilised as helpful risk factors for suspecting development of the condition[14-16].

On the basis of these assumptions, consideration should be given to screening for PPD, although evidence in support of universal screening tools is lacking. However, women with known risk factors for PPD may be selected for screening[17,18]. At present the most utilised tool for screening PPD is the Edinburgh Postnatal Depression Scale (EPDS) which consists in a 10-item self-rated questionnaire with a diagnostic cut-off of 10 (or greater) for possible PPD[19-22] even though the sensitivity and specificity varies across languages and cultures[23].

Even if many studies have clearly shown the high prevalence of depression and other mental disorders in CD, specific data on PPD in CD women are still lacking.

Aim of this study was to explore the prevalence of PPD in women suffering from CD compared to that recorded in healthy subjects.

MATERIALS AND METHODS

We performed a case-control study evaluating the prevalence of PPD in CD patients on gluten-free diet (GFD) in comparison with a control group of healthy subjects.

Between June 2010 and February 2013 we enrolled all coeliac women (Group A) followed-up at our Gastrointestinal Unit (tertiary centre for food intolerance and CD) who had given birth within the 8-wk period preceding their appointment at the Unit and had been on GFD for at least 1 year before pregnancy. CD patients were also classified in accordance with Oslo classification[1]. A group of consecutive healthy women who had given birth in the previous 8 wk were also recruited at two first-line obstetric Clinics as control group (Group B). The clinical interview was conducted postnatally by gastroenterologists not blinded on the clinical/pathological state of the subject. All women (CD patients and controls) with a diagnosis of active depression (based on DSM-IV criteria) formulated at least 3 mo before starting pregnancy and those already on treatment with anti-depressant drugs were excluded from the study.

A gynaecological evaluation explored menstrual cycle features, potential comorbidities, mode and outcome of delivery. Menstrual disorders were defined in presence of amenorrhea, dysmenorrhoea, pre-menstrual syndrome, polymenorrhea and oligo-menorrhea in accordance with the gynaecological literature[24,25]. All women underwent a conventional serological evaluation comprising the detection of tissue anti-transglutaminase antibodies level.

All participants were assessed blinded for PPD through clinical interview using the EPDS[19-22].

Furthermore, quality of life as defined through the SF-36 questionnaire was examined in all patients and controls[26-29].

All patients gave their written consent to participate in the study that was approved by local Ethical Committee.

Screening for PPD

EPDS is a 10-item (scored on a scale of 0-3) self-reported scale assessing the symptoms of PPD. This questionnaire has been validated also in Italy[30] so that we were able to use it for the population in our study with high values on diagnostic accuracy. EPDS is routinely used in many

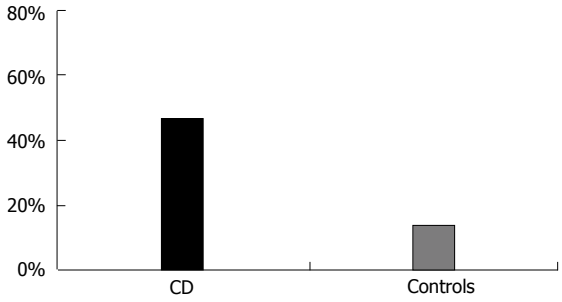

Figure 1 Prevalence (%) of positive Edinburgh Postnatal Depression Scale for post-partum depression in coeliac disease women and controls.

clinical services to screen for probable distress in women, both antenatally and postnatally, with a diagnostic cut-off ⩾ 10 (sensitivity 96%; specificity 92%)[31]. On these grounds, we established a cut-off ⩾ 10 as highly indicative of possible PPD. CD patients and controls with a EPDS ⩾ 10 were referred for psychiatric assessment. The screening for PPD was performed by an interviewer using the EPDS score with a ⩾ 10 cut-off and a clinical interview performed by an expert psychiatrist according to the DSM IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision).

SF-36 questionnaire

The SF-36 survey consists of a 36-item questionnaire that includes eight components: physical functioning, role limitations due to physical health, bodily pain, general health, vitality, social functioning, role limitations due to emotional and mental health issues. These eight domains form two broader health dimension scales: the physical (PCS) and mental (MCS) component scales. The SF-36 subscales and composite scores can be summarised through means ± SD, with higher scores indicating better health and well-being. Low scores on the PCS indicate limitations in physical functions and general health, and/or physical pain, while higher scores suggest no physical limitations, disabilities, or reductions in well-being. Similarly, low scores on the MCS suggest frequent experience of difficulties in psychosocial health, emotional problems and reduced vitality, while high scores indicate frequent positive affect and vitality, the absence of psychological distress and reduced or no limitations in daily social activities[26-29].

Sample size and statistical analysis

We estimated that a sample size of 67 participants would be able to offer a 80% power to detect a 20% difference between the two groups (when assuming a known PPD prevalence of 13% in general population)[10,11].

Statistical analysis was performed by using χ^2, Mann Whitney *U* test and odd ratio (OR) calculation when indicated; differences were considered significant with a $P < 0.05$. All statistical analyses were performed with software package SPSS for Windows (Rel SPSS 14.0; SPSS Chicago, IL).

Table 1 Demographic and serological features of patients with coeliac disease and controls

	Group A Celiac women (# 70)	Group B Healthy subjects (# 70)	*P* value
Mean age (yr)	33.32 ± 2.88	32.32 ± 4.32	0.7
Level of instruction (yr)	11.06 ± 3.54	11.30 ± 3.58	0.9
Hb (gr/dL)	11.03 ± 2.46	11.21 ± 2.54	0.7
Cholesterol (mg/dL)	156.85 ± 35.82	149.68 ± 37.02	0.2
Anti-transglutaminase IgA (U/mL)	< 0.1	< 0.1	0.9
Type of childbirth			
Delivery by caesarean section	36/70	31/70	0.7
Vaginal delivery	34/70	39/70	0.7
Outcome of birth			
Live birth	70/70	70/70	1.0
Congenital malformations	0/70	1/70	0.9
Number of pregnancies			
First pregnancy	34/70	37/70	0.7
Second pregnancy	25/70	24/70	0.9
> 2 pregnancies	11/70	9/70	0.8
Weeks of gestation	37.7 ± 1.78	37.95 ± 1.43	0.9

RESULTS

The study included 70 CD patients on GFD (group A) and 70 healthy controls (group B). According to the Oslo classification[1], CD patients were classified as classical CD in 31 cases (44%), non-classical CD in 34 subjects (49%), asymptomatic CD in 5 patients (7%). About symptoms of CD patients, 30 subjects (43%) showed anaemia, 31 (44%) had diarrhoea, 33 (47%) presented weight loss, 26 (37%) showed abdominal pain and 28 (40%) asthenia.

Group A and Group B resulted well matched for age (group A: 33.3 ± 2.9 years; group B: 32.3 ± 4.3 years; *P* = NS) and level of school education (group A: 11.1 ± 3.5; group B: 11.3 ± 3.9; *P* = NS).

No significant difference was evident between the two groups in terms of laboratory variables: haemoglobin (group A: 11.03 ± 2.46; group B: 11.21 ± 2.54; *P* = NS); cholesterol (group A: 156.85 ± 35.82; group B: 149.68 ± 37.02; *P* = NS); glycaemia (group A: 80.6 ± 10.3; group B: 79.81 ± 13.3; *P* = NS); alanine transaminase (group A: 25.41 ± 12.6; group B: 24.18 ± 11.9; *P* = NS). Demographic and serological features of patients and controls are reported in Table 1. The level of anti-transglutaminase antibodies was normal for all participants (CD patients on GFD and controls).

Thirty-three subjects in Group A and 10 in Group B had EPDS scores higher than the selected cut-off (47.1% of CD patients *vs* 14.3% of controls; $P < 0.01$; OR = 3.3). The Figure 1 shows the prevalence (%) of positive EPDS for PPD in CD women and controls. EPDS score was higher in CD women compared to the controls (mean score: group A 9.9 ± 5.9; group B 6.7 ± 3.7; $P < 0.01$). After psychiatric assessment, 29 out of the 33 patients in Group A (prevalence 41%) and 8 out of the 10 people in the control group (prevalence 11%) with high EPDS scores received a diagnosis of PPD. These results confirmed the high sensitivity of EPDS in our country

Table 2 Main results

	Group A Celiac women *n* = 70	Group B Healthy subjects *n* = 70	*P* value
EPDS score (mean ± SD)	9.94 ± 5.98	6.7 ± 3.73	< 0.01
Patients with EPDS score ⩾ 10 (%)	33/70 (47)	10/70 (14)	< 0.01
PPD after psychiatric assessment	29/33	8/10	0.06
SF-36			
PCS (mean ± SD)	55 ± 12	66 ± 8	0.03
MCS (mean ± SD)	43 ± 11	56 ± 7	0.02

EPDS: Edinburgh postnatal depression scale; PPD: Post-partum depression.

(sensitivity 86%). The majority of patients suffered from a mild form of PPD received only active psychological support; only 6 patients (16%) needed anti-depressants and long-term psychiatric follow-up. Results are reported in Table 2.

Regarding the circumstances of delivery, no significant differences were observed between the two groups in terms of type of delivery (group A: caesarean section delivery 51.4%; vaginal delivery 48.6%; group B: caesarean section delivery 44.3%; vaginal delivery 55.7%; P = NS) and birth outcomes (live birth: group A 100%; group B 100%; congenital malformations: group A 0%; group B 1.5%; P = NS). On the contrary, a significant association was observed between the onset of PPD and a previous menstrual disorder in women suffering from CD. Among these, 23 women with and only 7 without previous gynaecological diagnosis of menstrual disorders were positive for PPD at EPDS (69.7% *vs* 18.9%; $P < 0.001$; OR = 3.6); this association was not evident in the control group (25% *vs* 33%; $P = 0.4$).

With regard to quality of life as measured by the SF-36, outcomes were significantly better in controls than in patients with CD in terms of both PCS (55 ± 12 in Group A *vs* 66 ± 8 in Group B; $P = 0.03$) and MCS (43 ± 11 in Group A *vs* 56 ± 7 in Group B; $P = 0.02$). These outcomes were significantly and inversely correlated with the presence of PPD ($P = 0.02$).

DISCUSSION

The present study, focused on PPD, has shown a higher prevalence of this condition in women affected by CD on GFD when compared to healthy subjects (47% *vs* 14%); after psychiatric assessment, the prevalence of PPD was 41% in CD patients compared to 11% of control group. To our knowledge, this is the first paper reporting a higher incidence of this kind of psychiatric condition in CD.

Many studies have evaluated the psychiatric/mental aspects of CD[4,6,7]. In particular, a recent meta-analysis has clearly shown that depression is consistently more common and severe in adults with CD than in healthy adults[32]. After the diagnosis of CD, these patients must avoid foods containing grains (wheat, rye, and barley) for the rest of their life. From this point of view, depression in adult CD may represent a non-specific disorder precipitated by adverse physical symptoms along with personal and social limitations imposed by the chronic disease and the related dietary restrictions.

In effect, in terms of clinical depression, adults affected by CD do not differ substantially from those with other types of physical illness. In addition, many reports have underlined the high prevalence in patients with CD compared to healthy control groups of other psycho-pathological conditions, such as anxiety and sleep disorders[5,8,9].

In our study, a probable diagnosis of PPD identified on the basis of EPDS questionnaire scores was subsequently confirmed through psychiatric assessment in a majority of cases (sensitivity: 86%).

Although frequent, PPD was mild in most affected CD patients and was effectively treated by psychological support. This finding is in accordance with previous reports showing a high rate of missed diagnosis of PPD in the general population, especially if PPD presented in a mild form[33]. The screening approach led to early diagnosis and treatment in 43 women (33 CD, 10 controls) affected by PPD, potentially preventing negative consequences for mothers and their newborns.

The high prevalence of PPD in our CD population could be due to several causes. Firstly, PPD could be the expression of an underlying subclinical depression which develops features of PPD and clinical relevance during pregnancy or immediately after childbirth as a result of the heightened anxiety that can be typical of this period. This hypothesis is also in accordance with the low level of quality of life of CD patients compared to the controls[9,34]. On these bases, it is possible that a substantial proportion of the PPD cases were also mildly symptomatic during pregnancy so that the screening for and treating depression in CD patients during pregnancy might be more beneficial than waiting to screen them in the postnatal period. Many studies have highlighted the close relationship between low quality of life, anxiety and depression in patients with CD and, from this point of view, our findings are not surprising[4,9]. In addition, a woman suffering from CD may be concerned about the possible "genetic transmission" of CD to the newborn, thus worsening her anxiety.

Another explanation for our results could be found in inflammatory/autoimmune mechanisms. Many reports have investigated and underlined the possible role of inflammatory mediators in the pathogenesis of some variants of depression and of other mental problems such as sleep disorders[35,36]. Significant increase in pro-inflammatory cytokines and other inflammation-related proteins in major depression were found in plasma and cerebrospinal fluid. Furthermore, elevated levels of pro-inflammatory cytokines persist after clinical symptoms of depression are in remission and can also predict the onset of a depressive episode. Antidepressant treatment can lead to a normalization of elevated cytokine levels in major depression[35]. In effect, a recent meta-analysis has investigated the effect of some anti-depressants on the level of inflammatory cytokines. The results of this study

underlined that antidepressant treatment is able to reduce levels of IL-1β and possibly those of IL-6. Stratified subgroup analysis by class of antidepressant indicated that serotonin reuptake inhibitors may reduce levels of IL-6 and TNFα[36]. All these consideration could be considered an indirect demonstration of the pivotal role of inflammation in determining depression (and PPD) symptoms.

In our CD population the occurrence of PPD was significantly correlated with the presence of previous menstrual disorders[37]. This result could be the expression of a pre-existing underlying hormonal alteration which can also affect and/or contribute to the incidence of mental disorders[38,39].

Interestingly, a recent paper by Buttner *et al*[39] has highlighted the significant association between previous menstrual disorder and depression with a twofold increased risk of PPD in women with menstrual irregularities[40]. In our control population this kind of association was not evident, probably because of the small number of controls with PPD (10 patients). Nevertheless, one could postulate that this association may be related to the range of alterations characterising the spectrum of gynaecological/obstetric manifestations of CD[41,42]. Unfortunately, our work did not include investigations for the measurement of sexual and other hormones and this hypothesis remains therefore unanswered. On the other hand, no significant association was seen between PPD and type/outcome of delivery between the two groups of subjects. About this issue, our work confirmed once again the very high percentage of caesarean delivery performed in South of Italy[43,44].

Our work presents some limitations. First of all, we included all CD patients on GFD in our study; the full compliance to GFD was confirmed by the negative level of anti-transglutaminase antibodies in all CD patients. We decided to exclude patients with new/recent diagnosis of CD (who would be patients on free diet) because the majority of patients followed-up at our Centre consisted of individuals with previous diagnosis of CD and already on GFD. Hence, in order to recruit a homogenous population to the study, we decided to exclude patients who had recently been diagnosed with CD and were on free diet. However, thanks to the composition of our sample, we were able to document that PPD in patients suffering from CD is not directly related to active gluten ingestion. Further studies (ideally multicentre) are needed to define the prevalence of PPD in CD women on free diet (*e.g.*, diagnosed with CD during their pregnancy or immediately after childbirth).

We used healthy subjects as control group. This methodological approach is necessary to demonstrate the real increase of PPD in patients with CD compared to the general population; however, it does not determine whether the high prevalence of PPD is related to CD specifically or to a non-specific "illness status". In effect, as mentioned earlier[32], adults affected by CD do not differ substantially in terms of incidence of depression from those with other types of physical illness and the same could be true of PPD. In view of this, only a study directly comparing prevalence of PPD in patients with CD with that in patients suffering from other diseases (*e.g.*, inflammatory bowel diseases, rheumatologic diseases) could clarify this aspect.

However, the high prevalence of PPD in patients suffering from CD and the clinical relevance of this psychiatric condition justify the routine use of the EPDS questionnaire in women with CD who have recently given birth.

In conclusion, post-partum depression is a frequent condition in women affected by CD on GFD, particularly in those with history of menstrual disorders. We suggest screening for PPD in all women with CD for early detection and prompt treatment of this condition.

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COMMENTS

Background

Celiac disease (CD) is an autoimmune enteropathy gluten-related, characterized not only by gastrointestinal symptoms, but also by an increased prevalence of psychological symptoms and mental disorders, specially depression, anxiety and sleep disorders.

Research frontiers

Focused data on post-partum depression (PPD) in celiac women are still lacking.

Innovations and breakthroughs

In this case-control study, the authors evaluate, for the first time in the literature, the prevalence of PPD in CD patients on gluten-free diet compared to that of healthy subjects experiencing a recent delivery.

Applications

The authors demonstrated that PPD was present in 47.1% of CD women and in 14.3% of controls ($P < 0.01$; OR = 3.3). In the CD population the occurrence of PPD was significantly correlated with the presence of previous menstrual disorders. The authors suggest screening for PPD in all women with CD experiencing delivery for early detection and prompt treatment of this condition.

Terminology

PPD is a psychiatric disorder affecting women after pregnancy and leading to disruptions in maternal-infant interactions, and lower cognitive functioning and behavioural problems in children.

Peer review

In this manuscript, the authors present psychiatric disorders - showing higher incidence of PPD in patients with CD in comparison to healthy women controls. From the gastrological point of view this is a well written paper, but it should be evaluated by a psychiatrist to assess accuracy of psychological tests used for the assessment of depression.

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ORIGINAL ARTICLE

Retrospective Study

Hepatoma-derived growth factor expression as a prognostic marker in cervical cancer

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Abstract

AIM: To examine the association of hepatoma-derived growth factor (HDGF) expression with the prognosis of patients with cervical cancer of the uterus (CC).

METHODS: HDGF is a unique nuclear growth factor, and it may play an important role in the development and progression of carcinoma. HDGF expression in 88 CC patients aged 23 to 76 years (median, 54 years) was analyzed by immunohistochemistry. A rabbit polyclonal antibody against the C-terminal amino acids (aa 231-240) of the human HDGF sequence was used as primary antibody at a dilution of 1:5000. This specific anti-HDGF antibody was purified using C-terminal peptide-conjugated Sepharose columns. Staining of endothelial cells in the noncancerous areas of each specimen was used as an internal positive control. Samples with more than 80% of tumor cells showing positive immunoreactivity in both the nucleus and cytoplasm were regarded as HDGF index level 2, more than 80% positive immunoreactivity in either the nucleus or cytoplasm as level 1, and less than 80% in both the nucleus and cytoplasm as level 0. The chi-square test and Fisher's exact probability test were used to examine the relationship between HDGF expression and clinicopathologic parameters, and statistical significance was examined by the log-rank test. Multivariate analysis of factors related to survival was performed using Cox's proportional hazards regression model. Statistical significance was set at $P < 0.05$.

RESULTS: The five-year overall survival rate was 82.9%. Fourteen patients died due to tumors, nine of whom had tumor recurrence at 2-21 mo (median, 10 mo) after surgery. Tumor recurrence in five patients was determined at the time of the patients' deaths. Nineteen cases were regarded as HDGF index level 0, 11 as level 1, and 58 as level 2. Patients with level 2 expression showed higher rates of histological classification of keratinized squamous cell carcinoma

and adenosquamous carcinoma (44.8% of level 2 patients and 13.3% in levels 0 and 1), deep invasion (pT2-4 in 65.5% of level 2 patients, and 30.0% in levels 0 and 1), the presence of lymphatic invasion (50.0% in level 2, and 20.0% in levels 0 and 1), and the presence of lymph node metastasis (37.9% in level 2, and 6.7% in levels 0 and 1). Patients with an HDGF index of level 2 CC showed poorer 5-year overall survival rates than those with level 0 or 1 CC (74.0% and 100%, respectively, P = 0.0036). Univariate analysis revealed that histological classification (P = 0.04), depth of tumor invasion (P = 0.0001), vascular invasion (P = 0.004), and lymph node metastasis (P = 0.0001) were significant factors affecting overall survival in addition to HDGF expression. Multivariate analysis revealed HDGF expression level and lymph node metastasis as independent prognostic factors for overall survival (P = 0.0148 and P = 0.0197, respectively). The prognostic significance of HDGF was further analyzed in pT1 and pT2-4 patient groups, respectively. Among patients with pT1 CC, one the 39 analyzed patients died during the study, and no difference was observed among patients with HDGF index level 0, 1, or 2 CC. However, prognostic significance of the HDGF index was observed in the pT2-4 patient group, in which the mortality rates of patients with HDGF index level 2 CC and those with level 0 or 1 CC significantly differed (P = 0.0463).

CONCLUSION: The HDGF expression level is of prognostic significance in CC.

Key words: Hepatoma-derived growth factor; Prognosis; Cervical cancer; Immunohistochemical analysis; Multivariate analysis

Core tip: Hepatoma-derived growth factor (HDGF) is a unique nuclear growth factor, playing an important role in the development and progression of carcinomas. Prognostic importance of HDGF expression has been reported in several cancers. In the present study, HDGF expression in cervical cancer was examined by immunohistochemistry, showing increased HDGF expression as a marker of deep invasion, lymphatic invasion, and lymph node metastasis. In addition, HDGF expression was an independent prognostic factor for overall survival.

Song M, Tomoeda M, Jin YF, Kubo C, Yoshizawa H, Kitamura M, Nagata S, Ohta Y, Kamiura S, Nakamura H, Tomita Y. Hepatoma-derived growth factor expression as a prognostic marker in cervical cancer. *World J Obstet Gynecol* 2015; 4(1): 16-23 Available from: URL: http://www.wjgnet.com/2218-6220/full/v4/i1/16.htm DOI: http://dx.doi.org/10.5317/wjog.v4.i1.16

INTRODUCTION

Cervical cancer of the uterus (CC) is one of the most common carcinomas in females globally[1,2]. Human papilloma virus infection through sexual transmission is causative of CC carcinogenesis[3,4], and the rate of CC development in females of reproductive age is increasing worldwide[5,6]. More than 25% of patients with CC are under 40 years of age, and its occurrence in nulliparous women is increasing[7]. Due to the rise of mass screening, many cases of CC are detected at an early stage of the disease, and the number of curable patients is increasing[8]. Nevertheless, it is desirable to protect the fertility of nulliparous patients and patients of child-bearing age during treatment[5,6]. Thus, fertility-sparing surgeries, such as vaginal radical trachelectomy or abdominal radical trachelectomy, have been introduced[9,10].

Appropriate candidates for fertility-sparing surgery are patients with tumors classified by the International Federation of Gynecology and Obstetrics (FIGO) as stage IA1 with lymphovascular space involvement, IA2, and IB1 with tumors less than 2 cm in size[11]. In IB1 patients with tumors larger than 2 cm in size, higher risks of extrauterine spread and recurrence have been statistically demonstrated[12]. In cases in which lymph node metastasis is diagnosed or highly suspected, the surgery should be radicalized, or chemoradiotherapy should be undertaken instead[13].

Hepatoma-derived growth factor (HDGF) is a heparin-binding protein purified from the conditioned medium of the hepatocellular carcinoma (HCC) cell line HuH-7, which can proliferate autonomously in a serum-free chemically defined medium[14,15]. HDGF is an acidic 26 kDa protein consisting of 230 amino acids with no hydrophobic signal sequence in its N-terminus, and it has high affinity for the glycosaminoglycans heparin and heparan sulfate[16,17]. Exogenously supplied HDGF stimulates the proliferation of fibroblasts, endothelial cells, vascular smooth muscle cells, pulmonary epithelial cells and hepatocytes, as well as HCC, lung cancer and colon cancer cells, through the stimulation of ERK phosphorylation[18-20]. HDGF translocates to the nucleus, and this nuclear targeting stimulates cell proliferation[18].

Following these observations, we hypothesized that HDGF expression in human malignant tumors might influence metastasis and patient prognosis. Indeed, several reports have shown the correlation between increased HDGF expression and poor prognosis in cancers[21-24]. However, there have been no reports evaluating the correlation of HDGF expression with CC clinicopathologic features or prognosis.

In the present study, which included 88 patients with CC who were undergoing surgery, the expression levels of HDGF and the relationship between HDGF expression and clinicopathological features and prognosis were analyzed.

MATERIALS AND METHODS

Tissue specimens

Tumor tissue was collected from 88 patients with CC who underwent surgical resection between January 1995 and March 2002 at the Gynecology Department, Osaka Medical Center for Cancer and Cardiovascular Diseases,

Osaka, Japan. Patient ages ranged from 23 to 76 years (median, 54 years). Informed consent for the use of the specimens was obtained from all patients. The surgical procedures performed included cervical conization alone in two patients, total hysterectomy alone in five, total hysterectomy plus salpingo-oophorectomy in one, total hysterectomy plus salpingo-oophorectomy plus pelvic lymph node dissection in one, modified radical hysterectomy alone in three, modified radical hysterectomy plus salpingo-oophorectomy in two, modified radical hysterectomy plus pelvic lymph node dissection in one, modified radical hysterectomy plus salpingo-oophorectomy plus pelvic lymph node dissection in one, radical hysterectomy in six, radical hysterectomy plus salpingo-oophorectomy in 52, and radical hysterectomy plus salpingo-oophorectomy plus pelvic lymph node dissection in 14. Samples obtained from uterine lesions and dissected lymph nodes were fixed in 10% formalin and routinely processed for paraffin embedding. Histologic sections cut at a thickness of 4 μm were stained with hematoxylin and eosin, and immunoperoxidase procedures were performed [Avidin-Biotin Complex (ABC) method]. Histologic sections were reviewed by one of the authors (Tomita Y) to define the extent and mode of cancer invasion in the uterus as well as lymph node metastasis. Tumor stages were classified according to the FIGO and pTNM classification[25].

After surgery, all samples were examined by laboratory procedures, such as routine peripheral blood cell counts, and all patients underwent chest roentgenogram, computed tomography of the abdomen, colposcopic examination, and smear cytology at 6-12-mo intervals. Neoadjuvant chemotherapy was performed in nine patients. Adjuvant therapy was performed in 57 patients, radiotherapy alone in 26 patients, chemotherapy alone in 21, and combined chemo- and radiotherapy in nine. The chemotherapeutic protocols were as follows; fluorouracil (5-FU) or its derivative alone in six patients, cisplatinum (CDDP) or its derivative alone in 23, 5-FU and CDDP in one, and irinotecan in one. The follow-up period for survivors ranged from 12-145 (median, 88.2) mo. This study protocol was approved by the institutional review board of Osaka Medical Center for Cancer and Cardiovascular Diseases.

Immunohistochemical assays

Immunohistochemical studies were completed using the avidin-biotin-peroxidase complex method. Antigen retrieval was performed by heating the sections in 10 mmol/L citrate buffer (pH 6.0) for 5 min. A rabbit polyclonal antibody against the C-terminal amino acids (aa 231-240) of the human HDGF sequence was used as the primary antibody at a dilution of 1:5000. This specific anti-HDGF antibody was purified using C-terminal peptide-conjugated Sepharose columns[16,17]. Non-immunized rabbit IgG (Vector Labs; Burlingame, CA) was used as a substitute for the primary antibody to verify the possibility of false-positive responses due to the non-specific binding of IgG or the secondary antibody. Counterstaining was performed with hematoxylin.

Specimen classification based on immunohistochemical results

All immunohistochemically stained sections were blindly examined without any prior knowledge of the clinicopathologic parameters or patient outcome. Staining of endothelial cells in the noncancerous areas of each specimen was used as an internal positive control. Consistent HDGF expression in endothelial cells has been reported[19,20]. The HDGF expression pattern was independently evaluated in both the nucleus and cytoplasm; cells showing staining intensity similar to or stronger than that in the nucleus or cytoplasm of endothelial cells were regarded as nucleus positive or cytoplasm positive, respectively. Samples with more than 80% of tumor cells showing positive immunoreactivity in both the nucleus and cytoplasm were regarded as HDGF index level 2, more than 80% positive immunoreactivity in either the nucleus or cytoplasm as level 1, and less than 80% in both the nucleus and cytoplasm as level 0.

Statistical analysis

SAS software (Statistical Analysis System Institute, Cary, NC) was used for all statistical analyses. The chi-square test and Fisher's exact probability test were used to examine the relationship between HDGF expression and clinicopathologic parameters of prognosis. The cumulative survival rate was calculated by the Kaplan-Meier method, and statistical significance was examined by the log-rank test[26]. Multivariate analysis of factors related to survival was performed using Cox's proportional hazards regression model[27]. Statistical significance was set at $P < 0.05$.

RESULTS

Histologic findings

Histologically, 27 tumors were keratinizing squamous cell carcinoma, 60 were non-keratinizing squamous cell carcinoma, and one was adenosquamous carcinoma. Tumor cells invaded less than 5 mm (pT1a) in 14 patients, more than 5 mm but confined to the cervix (pT1b) in 27, beyond the uterus without parametrial invasion (pT2a) in 15, to the parametrium but not to the pelvic wall or lower third of the vagina (pT2b) in 28, to the pelvic wall or lower third of the vagina (pT3) in three, and to the mucosa of the bladder or rectum (pT4) in one. Sixty-four patients were node negative (pN0), and 24 had regional lymph node metastasis (pN1).

Patient outcome

The five-year overall survival rate was 82.9%. Fourteen patients died due to the tumors, nine of whom had tumor recurrence at 2-21 mo (median, 10 mo) after surgery. Tumor recurrence in five patients was determined at the time of the patients' deaths.

Expression of HDGF in CC

The HDGF staining pattern in CC varied; 65 cases (73.9%) showed strong staining in the nuclei of more than 80% of tumor cells and were thus regarded as nucleus positive, while 62 cases (70.5%) with strong cytoplasmic staining in

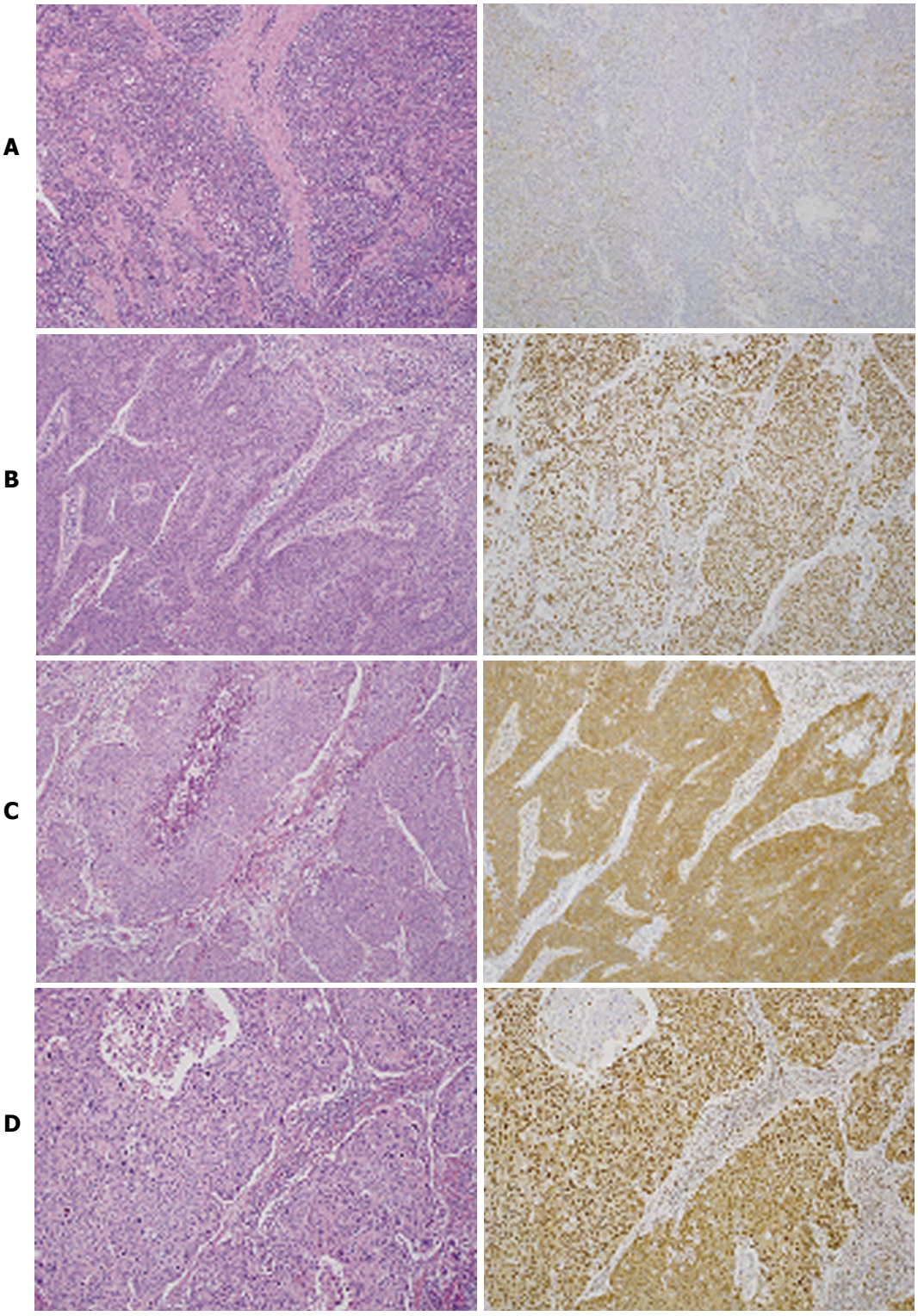

Figure 1 Hematoxylin and eosin staining (left lane) and hepatoma-derived growth factor immunohistochemistry (right lane). A: CC with HDGF index level 0. The majority of tumor cells were negatively stained for HDGF; B: CC with HDGF index level 1. More than 80% of tumor cells were positively stained in the nucleus, but negatively stained in the cytoplasm; C: CC with HDGF index level 1. More than 80% of tumor cells were positively stained in the cytoplasm, but negatively stained in the nucleus; D: CC with HDGF index level 2. More than 80% of tumor cells were positively stained in both the nucleus and cytoplasm (magnification × 50). CC: Cervical cancer of the uterus; HDGF: Hepatoma-derived growth factor.

more than 80% of tumor cells were cytoplasm positive. Fifty-eight cases (65.9%) determined as both nucleus and cytoplasm positive were regarded as HDGF index level 2. Eleven cases with HDGF expression either in the nucleus or the cytoplasm were classified as HDGF index level 1 and 19 others as HDGF index level 0 (Figure 1).

The relationship between clinicopathological features and HDGF expression in CC

The correlations between HDGF tumor expression

Table 1 Correlation of hepatoma-derived growth factor expression levels with clinicopathological factors in uterine cervical cancer patients *n* (%)

Factor	Category	No. of patients	HDGF expression			*P* value
			Level 0	Level 1	Level 2	
		n = 88	*n* = 19	*n* = 11	*n* = 58	
Age, yr		51.6 ± 13.8	46.7 ± 15.5	55.6 ± 12.1	52.4 ± 13.2	0.17
Histological differentiation	1: Keratinized SQ	29	2 (7)	2 (7)	25 (86)	0.037
	2: Nonkeratinized SQ	58	17 (29)	9 (16)	32 (55)	
	3: Adenosquamous carcinoma	1	0 (0)	0 (0)	1 (100)	
Lymphatic permeation	1: Absent	53	18 (34)	6 (11)	29 (55)	0.0006
	2: Present	35	1 (3)	5 (14)	29 (83)	
Vascular permeation	1: Absent	65	18 (28)	6 (9)	41 (63)	0.019
	2: Present	23	1 (4)	5 (22)	17 (74)	
Depth of invasion (pT)	1: T1	41	15 (37)	6 (15)	20 (49)	0.028
	2: T2	43	4 (9)	4 (9)	35 (81)	
	3: T3	3	0 (0)	1 (33)	2 (67)	
	4: T4	1	0 (0)	0 (0)	1 (100)	
Lymph node metastasis (pN)	1: Absent	64	12 (19)	9 (14)	36 (56)	0.0004
	2: Present	24	0 (0)	2 (8)	22 (92)	
Stage	1: Ⅰ	43	15 (35)	7 (16)	21 (49)	0.014
	2: Ⅱ	36	4 (11)	4 (11)	28 (88)	
	3: Ⅲ	5	0 (0)	0 (0)	5 (100)	
	4: Ⅳ	4	0 (0)	0 (0)	4 (100)	
Types of surgery	1: Conization	2	2 (100)	0 (0)	0 (0)	0.0001
	2: Total hysterectomy	7	7 (100)	0 (0)	0 (0)	
	3: Modified radical hysterectomy	7	4 (57)	0 (0)	3 (43)	
	4: Radical hysterectomy	72	6 (8)	11 (15)	55 (76)	
Salpingo-oophorectomy	1: Not performed	17	12 (71)	2 (12)	3 (18)	0.0001
	2: Performed	71	7 (10)	9 (13)	55 (77)	
Pelvic lymph node dissection	1: Not performed	71	14 (20)	9 (13)	48 (68)	0.7
	2: Performed	17	5 (29)	2 (12)	10 (59)	
Adjuvant/neoadjuvant chemotherapy	1: Not performed	53	16 (30)	5 (9)	32 (60)	0.03
	2: Performed	35	3 (9)	6 (17)	26 (74)	
Adjuvant radiotherapy	1: Not performed	52	16 (31)	9 (17)	27 (52)	0.003
	2: Performed	36	3 (8)	2 (6)	31 (86)	

SQ: Squamous cell carcinoma; HDGF: Hepatoma-derived growth factor.

and clinicopathological factors are shown in Table 1. In comparison to CC with HDGF index level 0 or 1, level 2 CC showed higher rates in the following categories: histological classification of keratinized squamous cell carcinoma and adenosquamous carcinoma (44.8% of level 2 patients and 13.3% in levels 0 and 1), deep invasion (pT2-4 in 65.5% of level 2 patients and 30.0% in levels 0 and 1), the presence of lymphatic invasion (50.0% in level 2 and 20.0% in levels 0 and 1), and the presence of lymph node metastasis (37.9% in level 2 and 6.7% in levels 0 and 1).

Uni- and multivariate analyses for the prognosis of patients with CC

Patients with HDGF index level 2 CC showed poorer 5-year overall survival rates than those with level 0 or 1 CC (74.0% *vs* 100%, $P = 0.0036$; Table 2, Figure 2A). In addition to HDGF expression, univariate analyses revealed that histological classification, depth of tumor invasion, vascular invasion, and lymph node metastasis were significant factors affecting overall survival (Table 2).

Multivariate analysis of factors significant in the univariate analyses revealed that HDGF index and lymph node metastasis were independent prognostic factors for overall survival (Table 3).

The prognostic significance of HDGF was further analyzed in pT1 and pT2-4 patient groups, respectively. Among patients with pT1 CC, one of the 39 analyzed patients died during the study, and no difference was observed among the groups. However, prognostic significance of the HDGF index was observed in the pT2-4 patient group, in which a significant difference in mortality was present between patients with HDGF index level 2 CC and those with level 0 or 1 CC (Figure 2B).

DISCUSSION

To identify optimal cutoff for the HDGF index in CC, statistical significance was examined at multiple cutoff levels, including < 50%, < 80%, < 90%, and < 100%. The prognostic significance was the greatest when the cutoff level was set at 80%. This categorization was therefore employed.

In the present study, CCs with HDGF index level 2 showed higher frequencies of deep tumor invasion (pT2-4), the presence of lymph node metastasis, and lymphatic invasion compared to level 1 and 2 cases, which are indicators of tumor progression in CC. In addition, patients with HDGF index level 2 CC showed a higher mortality rate compared with those with level 0 or 1. Previous studies have demonstrated that HDGF has a

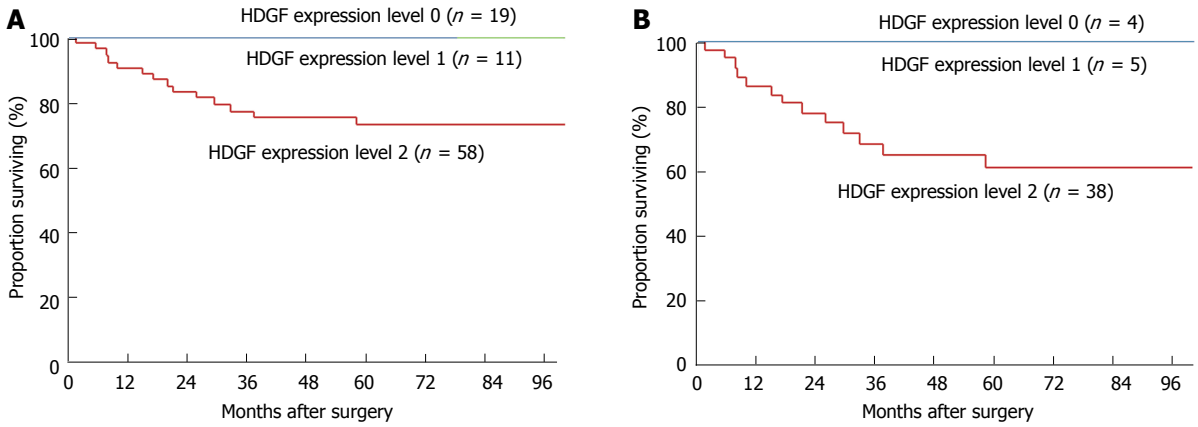

Figure 2 Overall survival curve of patients. A: Overall survival curve of patients with CC showing HDGF index levels 0, 1 and 2. Significant difference was observed between patients with HDGF index level 2 and other expression levels ($P < 0.0001$); B: Overall survival curve of patients with pT2-4 CC showing HDGF index levels 0, 1 and 2. Significant difference was observed between patients with HDGF index level 2 and other expression levels ($P = 0.0463$). CC: Cervical cancer of the uterus; HDGF: Hepatoma-derived growth factor.

Table 2 Univariate analysis of overall survival with clinicopathological factors in uterine cervical cancer patients

Factor	Category	No. of patients *n* = 88	No. of deaths *n* = 14	5-yr overall survival rate	*P* value
Age (yr)	1: ≤ 50	37	4	89%	0.27
	2: > 50	51	10	79%	
Histological differentiation	1: Keratinized SQ	29	5	81%	0.04[a]
	2: Nonkeratinized SQ	58	8	85%	
	3: Adenosquamous carcinoma	1	1	0%	
Lymphatic permeation	1: Absent	53	6	88%	0.15
	2: Present	35	8	75%	
Vascular permeation	1: Absent	65	6	90%	0.005
	2: Present	23	8	63%	
Depth of invasion (pT)	1: T1	41	1	97%	0.001[b]
	2: T2	43	10	75%	0.0001[d]
	3: T3	3	2	0%	
	4: T4	1	1	0%	
Lymph node metastasis (pN)	1: Absent	64	2	97%	0.0001
	2: Present	24	12	49%	
Stage	1: I	43	2	95%	0.0001[d]
	2: II	36	4	88%	
	3: III	5	4	20%	
	4: IV	4	4	0%	
Hepatoma-derived growth factor expression	1: Level 0	19	0	100%	0.0036[a]
	2: Level 1	11	0	100%	
	3: Level 2	58	14	74%	

[a]*P*: 1 and 2 *vs* 3; [b]*P*: 1 *vs* 2-4; [d]*P*: 1 and 2 *vs* 3 and 4. SQ: Squamous cell carcinoma.

Table 3 Multivariate analysis of overall survival with clinicopathological factors in uterine cervical cancer patients

Factor	Category	χ^2 value	*P* value
Histological differentiation	1: Keratinized and nonkeratinized squamous cell carcinoma 0: Adenosquamous carcinoma	0.51	0.474
Vascular permeation	1: Absent 0: Present	0.16	0.686
Depth of invasion (pT)-A	1: T1 0: T2-4	1.19	0.275
Depth of invasion (pT)-B	1: T1 and 2 0: T3 and 4	3.06	0.0801
Lymph node metastasis (pN)	1: Absent 0: Present	5.43	0.0197
Hepatoma-derived growth factor expression	1: Level 0 and 1 0: Level 2	5.94	0.0148

range of biological functions, including DNA synthesis, proliferation, growth stimulating activity, and vascular development[17-20]. The present study clearly indicates that HDGF is also involved in CC progression.

Fourteen of the 56 patients with HDGF level 2 expression died, whereas no deaths were observed among the 30 patients with HDGF level 0/1 expression. Although the distribution of patients was biased, the present results clearly indicate that HDGF expression is a useful marker to detect patients with CC who have a favorable prognosis. Indeed, multivariate analyses revealed the HDGF expression level to be an independent prognostic factor for CC. Analysis of the HDGF index together with other independent prognostic factors such as lymphatic invasion, vascular invasion, and lymph node metastasis might be a useful tool in predicting prognosis and determining appropriate therapeutic modalities for patients with CC.

Although patients with HDGF index level 0 or 1 can expect a favorable outcome after surgery, the occurrence of lymph node metastasis varied between the two groups. As patients with HDGF index level 0 CC showed no lymph node metastasis, they may suitable for fertility-sparing surgery. In contrast, patients with HDGF index level 1 or 2 exhibited a higher risk of lymph node metastasis; thus, standard surgeries such as radical hysterectomy are more preferable when the FIGO stage is higher than IA.

In conclusion, HDGF expression, as determined by immunohistochemistry, could be used as a new prognostic factor for CC. Although further study is still needed to elucidate the precise role of HDGF in CC malignancy, our results imply that HDGF may be a useful tool for determining the appropriate treatment of CC.

COMMENTS

Background

Cervical cancer of the uterus (CC) is one of the most common carcinomas in females, and its occurrence in nulliparous women is increasing. Fertility-sparing surgeries have been introduced to these patients, therefore assessment of histological grading of tumor is important. Expression of Hepatoma-derived growth factor (HDGF) in human malignant tumors might influence metastasis and patient prognosis, and reports have shown the correlation between increased HDGF expression and poor prognosis in several cancers, however, there have been no reports evaluating the correlation of HDGF expression and clinicopathologic features and prognosis of CC.

Research frontiers

HDGF expression as determined by immunohistochemistry could be used as a new prognostic factor for CC. Although further study is still needed to elucidate the precise role of HDGF in CC malignancy, the present report might imply that HDGF may be a useful tool in determining appropriate treatment of CC.

Innovations and breakthroughs

Fertility-sparing surgery, such as vaginal radical trachelectomy or abdominal radical trachelectomy, are introduced to patients with CC with the International Federation of Gynecology and Obstetrics (FIGO) stage IA1 with lymphovascular space involvement, IA2, and IB1 with tumors less than 2 cm in size. In cases where lymph node metastasis is diagnosed or highly suspected, the surgery should be radicalized, or chemoradiotherapy should be undertaken instead. The present study showed the correlation between HDGF expression and lymph node metastasis, then HDGF immunohistochemistry might be involved in the decisionmaking of CC treatment.

Applications

Patients with HDGF index level 0 CC showed no lymph node metastasis, they are suitable for fertility-sparing surgery. In contrast, patients with HDGF index level 1 or 2 have higher risk of lymph node metastasis; thus standard surgeries such as radical hysterectomy is more preferable when the FIGO stage is higher than IA.

Terminology

HDGF: a heparin-binding protein purified from the conditioned medium of the hepatocellular carcinoma (HCC) cell line HuH-7, which can proliferate autonomously in a serum-free chemically defined medium. HDGF is an acidic 26 kDa protein consisting of 230 amino acids with no hydrophobic signal sequence in its N-terminus, and it has high affinity for the glycosaminoglycans heparin and heparan sulfate. Exogenously supplied HDGF stimulates the proliferation of fibroblasts, endothelial cells, vascular smooth muscle cells, pulmonary epithelial cells and hepatocytes, as well as HCC, lung cancer and colon cancer cells, through the stimulation of extracellular signal-regulated kinase phosphorylation. HDGF translocates to the nucleus, and this nuclear targeting stimulates cell proliferation.

Peer review

The author gives information about the expression levels of HDGF in 88 patients with cervical cancer undergoing surgery, and the relationship between HDGF expression and clinicopathological features and prognosis. The research group has great experiences in this area.

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REVIEW

Pioneering drugs for overactive bladder and detrusor overactivity: Ongoing research and future directions

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Abstract

The ongoing research on pioneering drug candidates for the overactive bladder (OAB) aimed to overcome the limitations of currently licensed pharmacotherapies, such as antimuscarinics, β3-adrenergic agents, and botulinum neurotoxin, has been reviewed performing a systematic literature review and web search. The review covers the exploratory agents alternative to available medications for OAB and that may ultimately prove to be therapeutically useful in the future management of OAB patients based on preclinical and early clinical data. It emerges that many alternative pharmacological strategies have been discovered or are under investigation in disease-oriented studies. Several potential therapeutics are known for years but still find obstacles to pass the clinical stages of development, while other completely novel compounds, targeting new pharmacological targets, have been recently discovered and show potential to translate into clinical therapeutic agents for idiopathic and neurogenic OAB syndrome. The global scenario of investigational drugs for OAB gives promise for the development of innovative therapeutics that may ultimately prove effective as first, combined or second-line treatments within a realistic timescale of ten years.

Key words: Detrusor overactivity; Drug therapy; Lower urinary tract symptoms; Overactive bladder; Urinary incontinence

Core tip: The forefront of global research scenario of investigational drug candidates for the management of patients with overactive bladder and detrusor overactivity was reviewed. Among a huge amount of exploratory compounds with completely new mechanisms of action, some promising pharmacological principles show potential to translate into novel therapeutics to be clinically used as first-line alternative treatments, or in combination with established drugs, or as second-line treatments in refractory patients.

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INTRODUCTION

Overactive bladder (OAB), defined as urinary urgency with or without urgency urinary incontinence, usually associated with increased urinary frequency and nocturia[1], is a very bothersome and debilitating chronic condition that severely affects the patient's quality of life[2]. The socioeconomic burden is very high due to the aging population, the OAB-associated comorbidities and the increased risk of hospitalization[3]. The pathophysiology is largely unknown, although multiple causes have been proposed, such as a primary detrusor dysfunction, observed as detrusor overactivity (DO) during urodynamic studies, an overactivity of the afferent arm of the micturition reflex, a primary dysfunction of higher central nervous system (CNS) inhibitory centers[4]. OAB is underdiagnosed and undertreated, however, the increase in patient awareness, the rise in the geriatric population, and the availability of more pharmacological principles have triggered a significant growth in the OAB market with a estimated market size of over $2 billion in 2012.

Pharmacological treatment has been based for years on antimuscarinic agents, but recently other two pharmacological principles have been approved for OAB by the United States Food and Drug Administration (FDA): the first β3-adrenergic agent, mirabegron (Myrbetriq®, Astellas, approved in June 2012), and the botulinum neurotoxin (Botox®, Allergan, approved in January 2013)[5,6]. Although many novel antimuscarinic and β3-adrenergic agents, and alternatives to the botulinum neurotoxin are under development, the ideal medication for the cure of OAB with an optimal profile in terms of safety, tolerability and efficacy is still to be discovered. A huge amount of preclinical studies is ongoing exploring the therapeutic potential of many novel compounds some of which already advanced to the clinical phases of development, which mixed results[7,8].

This review provides an extensive update on the exploratory drugs, alternative to available medications for OAB that may ultimately prove to be therapeutically useful in the future treatment of lower urinary tract symptoms (LUTS) and OAB.

LITERATURE RESEARCH

A systematic literature review search of peer-reviewed English-language full papers published by November 2014 has been performed. Medline databank was searched employing both "MeSH" and "free text" protocols, and using a combination of the following search terms: "urinary bladder, overactive", "urinary incontinence, urge", "lower urinary tract symptoms" AND "drug therapy". Scopus and ISI Web of Science databanks were also searched using the same search terms. A search of articles related to each specific compound was also performed. A hand search of reference lists of retrieved articles was performed in order to identify further studies not captured by the above used terms. Clinical trials and pharmaceutical companies' websites were also searched for pipeline projects. All the investigational pharmacological principles with at least preclinical evidence of activity against OAB/DO have been discussed.

DRUGS ACTING ON INHIBITORY CENTRAL MECHANISMS

GABA$_B$-receptor agonist

Compounds with agonist activity on γ-aminobutyric acid (GABA) receptors in the CNS exploit the inhibitory effect of this neurotransmitter on micturition reflex[9]. Baclofen, a GABA$_B$-receptors agonist, is used for the treatment of neurological spasticity, particularly in the lower limb. Baclofen is pumped directly into the subarachnoid space by means of a programmable pump *via* a catheter system. Preclinical studies showed that intrathecal baclofen was effective in attenuating oxyhemoglobin-induced DO[10] and, in rats with spinal cord injury (SCI), produced a dose-dependent inhibition of non-voiding bladder contractions and a decrease in micturition pressure[11]. Clinical studies based on urodynamic evaluations showed that the continuous intrathecal baclofen pump infusion is effective in the management of patients with medically-refractory neurogenic DO (NDO) and decreased bladder compliance[12]. Although baclofen gained approval for treatment of NDO in SCI patients, the narrow therapeutic window and the tolerability profile limited its widespread clinical use. ADX71441, a novel, potent and selective agonist of GABA$_B$-receptor, showed efficacy in rodent models of OAB and may allow further exploitation of this central inhibitory mechanism[13].

Anticonvulsants

The inhibition of the GABA transporters (GAT) that are thought to provide a GABA inactivation mechanism in the CNS, has been explored as a possible pharmacological principle aimed to treat DO. Tiagabine, an anticonvulsants that selectively inhibits the GABA re-uptake *via* the GABA transporter GAT1, given intravenous or intrathecal in rats, improved storage phase parameters suggesting a potential utility for OAB treatment[14].

Gabapentin is a putative GABA analog crossing the blood-brain barrier originally developed to treat epilepsy, and currently used for neuropathic pain and other conditions. Its mechanism of action has not been fully elucidated although it appears to have inhibitory

activity on afferent C-fibers likely by binding to α-2-delta subunit of voltage-dependent calcium channel[15]. Carbone *et al*[16] reported in a pilot study that gabapentin improved both symptoms and urodynamic parameters in NDO patients. Kim *et al*[17] reported that the drug was well tolerated and improved symptoms in 14 out of 31 antimuscarinics-refractory OAB patients. Recently, beneficial clinical effects of gabapentin as an add-on therapy have been reported also in 16 out of 30 children with antimuscarinics-refractory OAB[18]. Phase Ⅱ trials are ongoing comparing gabapentin with solifenacin in OAB patients[19] and evaluating the efficacy and tolerability of a combination of low doses of gabapentin and oxybutynin[20].

Pregabalin (Lyrica®, Pfizer) is an anticonvulsant drug mainly used for neuropathic pain and fibromyalgia. Like gabapentin, pregabalin binds to the α-2-delta subunit of the voltage-dependent calcium channel in the CNS, leading to decreased release of several neurotransmitters[21]. A phase Ⅱ trial is ongoing in patients with idiopathic OAB comparing pregabalin with tolterodine and their combination[22]. Furthermore, preclinical data showed significant improvement on the urodynamic parameters of an animal model of NDO providing a rationale for future proof-of-concept clinical trial on NDO patients[23].

Levetiracetam is an antiepileptic drug with a mechanism of action not yet clarified, although the drug binds to the synaptic vesicle glycoprotein SV2A and inhibits presynaptic calcium channels reducing neurotransmitter release and acting as a neuromodulator[24]. Experimental findings in spinal cord transected rats have shown that levetiracetam, administered by subcutaneous osmotic minipump, improved urodynamic parameters in this animal model of NDO[25].

GABAergic gene therapy

An alternative strategy is based on increasing GABA in the spinal cord *via* viral-mediated gene delivery. Injection in SCI rats of HSV-GAD (replication-defective herpes simplex virus vectors encoding genes of glutamic acid decarboxylase, the GABA synthesis enzyme) significantly decreased the number and amplitude of non-voiding contractions compared with controls, without blunting micturition pressure likely *via* the inhibition of the afferent limb of the micturition reflex[26]. Thus, *GAD* gene therapy gives promise to become a novel therapy of urinary dysfunctions in SCI patients.

Glycinergic drugs

Glycine is a major inhibitory neurotransmitter in the spinal cord. Animal studies suggest that glycinergic neurons have an important inhibitory effect on the spinobulbospinal micturition reflexes at the level of the lumbosacral cord[9].

The extracellular concentration of glycine at synapses is regulated by two types of glycine transporters (GlyTs): GlyT1 and GlyT2[27]. In rats, GlyT2 plays a major role in the clearance of extracellular glycine in the spinal cord and its inhibition leads to amelioration of cyclophosphamide-induced DO and pain behavior[28]. As a result, activation of glycinergic inhibitory mechanisms by GlyT2 inhibitors has been suggested as a novel therapeutic strategy for OAB and bladder pain syndrome.

DRUGS ACTING ON MONOAMINES RECEPTORS

Inhibitors of monoamine-reuptake

Inhibitory effects on micturition are known side-effects of drugs with inhibitory action on the monoamine reuptake, including tricyclic antidepressants. Furthermore, depression is more common in patients with OAB and a shared deficiency of monoamine (serotonin and noradrenaline) behind both depression and OAB has been suggested[29].

Imipramine, a tricyclic antidepressants, improves storage LUTS and DO at the cost of not negligible side-effects. Antidepressants selective serotonin reuptake inhibitors, such as escitalopram, are under evaluation for efficacy in OAB patients[30].

Duloxetine, an antidepressant acting as a selective serotonin-norepinephrine reuptake inhibitor (SNRI) and approved for the treatment of stress urinary incontinence for its stimulatory activity on external urethral sphincter, demonstrated significant efficacy compared to placebo in relieving urinary symptoms in women with OAB[31]. However, the side-effects of this compound significantly limit patient's compliance.

Based on animal experiments showing that besipirdine, a SNRI that interacts also with α1 (agonist) and α2 (antagonist) receptors, significantly and dose-dependently improves storage function and external urethral sphincter activity[32], a human proof-of-concept study has been initiated by UroGene in patients with storage LUTS[33].

Serotonin receptors agonists

Increasing evidence indicates that serotonin [5-hydroxytryptamine (5-HT)] is involved in a complex way in the control of micturition at central and peripheral sites, with both inhibitory and facilitatory effects[34-41], although the serotonergic pathway generally enhances urine storage by facilitating the vesical sympathetic reflex pathway and inhibiting the parasympathetic voiding pathway.

The 5-HT_{1A} receptor agonist 8-OH-DPAT has been investigated in alpha-chloralose anesthetized or conscious chronic SCI cats[37]. This compound significantly increased the bladder volume threshold for eliciting a large amplitude micturition contraction, but only slightly reduced the amplitude of the contractions, indicating that drugs that activate 5-HT_{1A} receptors might be useful in treating NDO after SCI. 8-OH-DPAT also improved voiding efficiency and maximum intravesical pressure, and enhanced the external urethral sphincter

tonic and bursting activity in a rat model of incomplete cauda equina/conus medullaris injury[38].

5-HT$_2$ and 5-HT$_3$ receptors mediate excitatory effects on sympathetic and somatic reflexes to increase outlet resistance, and preclinical studies have shown that 5-HT$_{2C}$ and 5-HT$_3$ receptors play an inhibitory role on micturition reflex suggesting that agonists at this site may have potential as candidate drugs for OAB[36].

Serotonin receptors antagonists

The peripheral excitatory function of serotonin is increased in disorders known to be associated with DO, such as bladder outlet obstruction (BOO) and diabetes[39]. The facilitatory action on micturition reflex of the 5-HT$_{2A}$ receptor has been demonstrated in rats and its overexpression observed in BOO rat bladder[36]. Accordingly, sarpogrelate (a 5-HT$_{2A}$ selective antagonist) counteracted in diabetic rat bladder the increased contractile response to 5-HT in a dose-dependent manner[39]. Accordingly, Takimoto *et al*[40] reported a symptomatic benefit in patients with diabetes and refractory OAB treated with sarpogrelate.

5-HT$_4$ and 5-HT$_{1A}$ receptors have been also involved in micturition control and their selective antagonists such as piboserod and WAY100635, respectively, potently inhibited the micturition reflex in animal models and human detrusor[34,41]. However, disappointing results have been reported with Rec-0545, a potent and selective antagonist of the 5-HT$_{1A}$ receptor evaluated by Recordati in a proof-of-concept trial for the treatment of OAB patients[42]. The combination of WAY100635 with duloxetine has been evaluated in a cat model of DO with promising results[43].

5-HT$_3$ receptor is another candidate target for the development of novel drugs for the OAB according to recent preclinical findings[44]. Dynogen Pharmaceuticals, Inc. is developing a drug (DDP225) with both 5-HT$_3$ receptor-antagonist and noradrenaline reuptake inhibitor properties for the treatment of OAB in patients who are not incontinent[45].

PURINERGIC RECEPTORS ANTAGONISTS

Several pharmacological approaches have been driven for a more in depth understanding of the physiology of the "mucosal bladder network" (the functional unit consisting of the urothelium, interstitial cells and afferent nerves) (Figure 1). An interesting hypothesis-driven approach for the future treatment of OAB is represented by the antagonism of purinergic receptors, namely P2X$_1$ and P2X$_3$/P2X$_{2/3}$[46]. Thus, several studies suggested that the adenosine 5'-triphosphate (ATP) and purinergic ionotropic (P2X) receptors are involved in DO[47-49]. This is not surprising taking into account that purinergic transmission has been found on both afferent and efferent signalling pathways within the lower urinary tract and appears to be abnormally enhanced with aging[50] and DO[51]. In particular, P2X3 receptors on sensory nerve terminals are involved in voiding dysfunctions of conscious chronic SCI rats, raising the possibility that P2X3 receptor antagonists might be useful for the treatment of NDO[46]. In human bladders with DO an increase in P2X3 receptor expression was observed[52].

The growing appreciation for the role of purinergic receptors in mediating nociceptive neurotransmission prompted the development of P2X receptor-selective antagonists as potential therapeutics for pain management[53]. The novel P2X3/P2X2/3 receptor antagonists possess attributes that offer the potential for optimization into candidate drug molecules not only for inflammatory and painful bladder conditions but also for OAB, in particular the recently developed RO3 compound (Roche Palo Alto) and the AF-742 (Afferent Pharmaceuticals), which is ongoing a phase Ⅱ trial for bladder pain syndrome[54]. Finally, P2X3 antisense oligonucleotides and RNA interference-mediated treatment, that appear to be promising for neuropathic pain management, might open up new avenues for therapeutic OAB strategies[55].

NEUROKININ RECEPTORS ANTAGONISTS

Substance P (SP) and neurokinin A (NKA) are neuropeptides with the highest affinity for NK1 and NK2 receptors, respectively. NK-receptors have been demonstrated in CNS regions involved in micturition control[56]. Many experimental observations are available indicating that spinal and supraspinal NK1 and NK2 receptors may modulate the micturition reflex[57-59].

Tachykinins are also released from urothelial/suburothelial capsaicin-sensitive afferents and are able to stimulate muscle tone and bladder contractions (NKA > NKB > SP), and to influence vascular tone and permeability ("neurogenic inflammation")[60,61]. Intravenous NK1 and NK2 receptor selective antagonists reduced DO in rat with SCI[61,62]. Perfusion of bladder with a NK1 receptor antagonist improved DO in rats wit cyclophosphamide-induced cystitis[48].

Mixed clinical results have been reported on some compound in this class. Aprepitant (Merck Sharp and Dohme Corp.) is a CNS-penetrating NK-1 antagonist used to treat chemotherapy-induced nausea. A pilot, proof-of-concept randomized controlled trial (RCT) including 125 post-menopausal women with urge or mixed (urge-predominant) incontinence reported satisfactory tolerability and efficacy of aprepitant over placebo in ameliorating OAB symptoms[63]. Serlopitant (MK0594, Merck Sharp and Dohme Corp.) has been evaluated in a RCT and, although it significantly decreased the primary endpoint of daily micturitions, no advantages in efficacy have been found *vs* tolterodine[64]. Netupitant (by Helsinn Healthcare) is another potent and selective NK1 receptor antagonist that failed to demonstrate superiority over placebo in a phase Ⅱ trial[65].

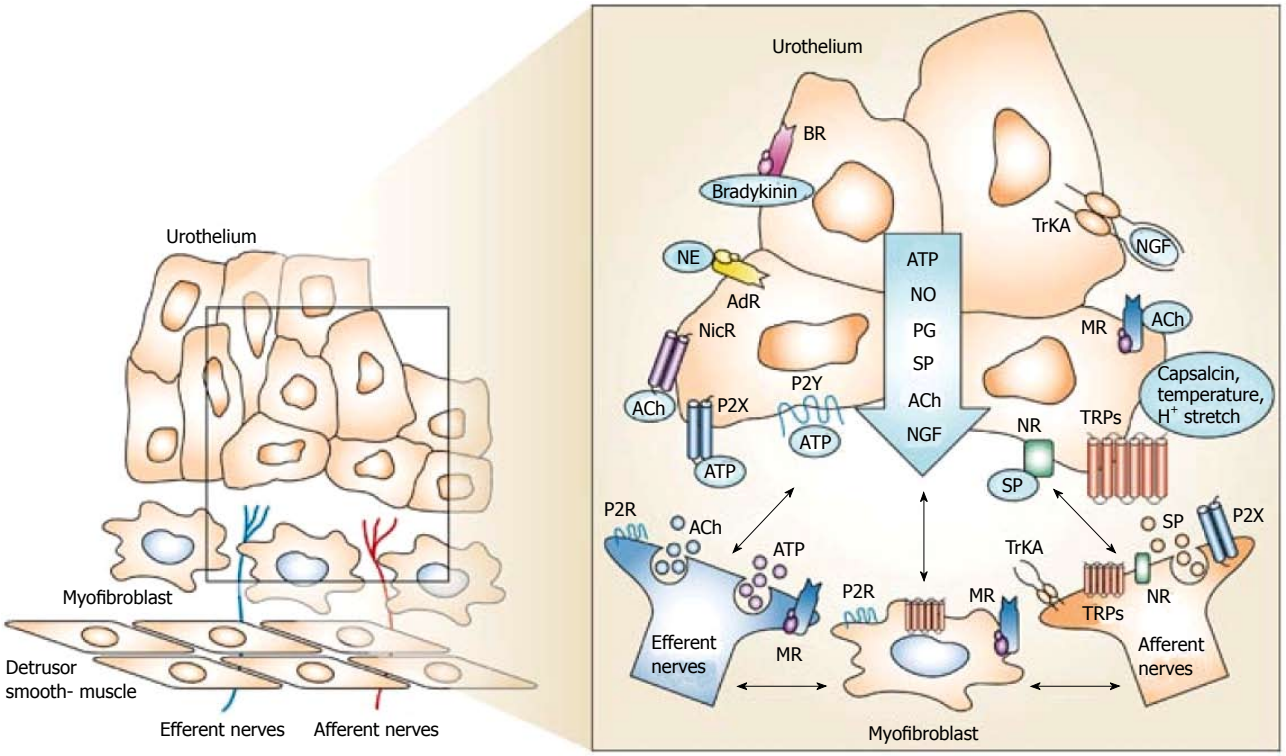

Figure 1 Hypothetical model that depicts possible interactions between bladder afferent and efferent nerves, urothelial cells, smooth muscle and myofibroblasts (interstitial cells). Stimulation of receptors and channels on urothelial cells can release mediators that target bladder nerves and other cell types; urothelial cells can also be targets for neurotransmitters released from nerves or other cell types. Urothelial cells can be activated by either autocrine (*i.e.*, autoregulation) or paracrine (release from nearby nerves or other cells) mechanisms (from Birder LA and de Groat WC[67], with permission of Nature Publishing Group). Ach: Acetylcholine; AdR: Adrenergic receptor; BR: Bradykinin receptor; MR: Muscarinic receptor; NE: Norepinephrine; NGF: Nerve growth factor; NR: Neurokinin receptor; NicR: Nicotinic receptor; NO: Nitric oxide; P2R: Purinergic 2 receptor unidentified subtype; P2X and P2Y: Purinergic receptors; PG: Prostaglandin; SP: Substance P; Trk-A: Receptor tyrosine kinase A, high affinity receptor for nerve growth factor; TRPs: Transient receptor potential channels.

VANILLOIDS AND TRANSIENT RECEPTOR POTENTIAL VANILLOIDS-ANTAGONISTS

Several "Transient receptor potential" (TRP) neuro-receptors have been involved in nociception and mechanosensory transduction in various organ systems as well as in storage bladder function and DO, offering the possibility to target bladder dysfunctions at the level of sensory signal initiation (Figure 1)[66].

"Transient receptor potential vanilloids 1" (TRPV1) is the principal transduction channel for nociception. TRPV1 is also found in myelinated Aδ-fibres and sensory unmyelinated C-fibres located in the pelvic nerve afferents and in a sub/intraurothelial plexus; it is sensible to bladder filling, bladder contractions and noxious stimuli[67]. TRPV1 is expressed also by the urothelial cells themselves[67].

C-fibres are normally silent but have been found to become active and to convey signal to the spinal cord in pathological situation such as OAB, NDO and SCI, resulting in the bothersome sensation of urinary urgency[68].

"Vanilloids" such as capsaicin are the best-known natural TRPV1 agonists and several trials showed that, given intravesically, they could cause a sustained activation of the TRPV1 receptor resulting in a desensitization of C-fibers with beneficial effects in patients with neurogenic or idiopathic DO, but at the cost of nonnegligible side-effects[69,70]. Resiniferatoxin is at least as effective as capsaicin, without the local side-effects although formal RCTs are needed to determine its appropriate use and dosage[71,72].

Potent orally-available small-molecule TRPV1 antagonists are undergoing clinical trials for chronic pain, but the lack of bladder-selectivity and potential effects on thermoregulation may be serious barriers for the clinical development[73,74]. XEN-D0501 (Provesica Ltd.) is a highly potent oral TRPV1 antagonist that was found to improve storage bladder function and reduce the intensity of capsaicin-induced bladder contractions in animal models; a phase Ⅰ study reported a satisfactory tolerability and safety[75]. XEN-D0501 is currently being assessed for efficacy in OAB in an international phase Ⅱ dose-ranging trial. JTS-653 (Japan Tobacco), MCP-101 (Mt. Cook Pharma) and SAF312 (Novartis Pharmaceuticals)[76], are other compounds in this class under investigation for the treatment of NDO.

Other TRP channels are expressed in the lower urinary tract (TRPV2, TRPV4, TRPM8, and TRPA1), and based on recent preclinical observations, TRPA1[77,78] and TRPV4[79-82] appear to have a critical role in bladder storage function and overactivity. Selective antagonists

for these ion channels are already available making the superfamily of TRP channels a very interesting class of potential targets for drugs aimed to treat LUTS/OAB/DO.

OPIOIDS

μ-opioid receptor-agonists

μ-opioid receptor (MOR) agonists are known for decades for their analgesic efficacy and excellent tolerability. Tramadol, an effective and safe analgesic, is a weak MOR-agonist, but its metabolites have a stronger MOR-agonist effect and also inhibit the reuptake of noradrenaline and 5-HT and elicit effects by indirectly activating serotonergic and α2-adrenergic receptors[83]. Promising clinical results in OAB patients were published on tramadol by Safarinejad *et al*[84] but the study has been retracted due to statistical errors. Singh *et al*[85] evaluated urodynamic effects of epidural tramadol in 15 subjects reporting that it increased bladder capacity and compliance and delayed filling sensations without affecting voiding phase, even for those with BOO.

Tramadol-like compounds with less incidence of nausea might have a treatment potential in patients with NDO and the development of novel MOR-agonists is ongoing. KN203 (KeyNeurotek Pharma) is the first compound of this class to be developed against OAB, and the results of a proof-of-concept study are expected to clarify its role in this clinical setting[86].

δ-receptor agonists

A growing volume of information supports a role for the δ-receptor in the regulation of bladder activity[87]. In contrast to μ-agonists, δ-*receptor* agonists present with lower toxicity and no addiction, their most crucial safety aspect being the incidence of seizure-like convulsions in rodents. MCP-202 is a compound in this class and in the development pipeline of Mt Cook Pharma for the treatment of OAB. A novel nonpeptide, orally bioavailable δ-receptor agonist (DPI-221) with satisfactory safety profile and high potency in extending micturition interval in mice has been recently developed[88].

NOP RECEPTOR AGONISTS

Nociceptin or orphanin FQ (N/OFQ) is the endogenous ligand of opioid-like receptor-4 (or NOP receptor)[89]. N/OFQ has a variety of effects both in the CNS and peripherally and there is evidence suggesting that N/OFQ inhibits the micturition reflex in rats by acting on the afferent bladder signalling and on supraspinal micturition sites[60,90], although a peripheral excitatory effect was also detected[90].

Lazzeri *et al*[91] reported that N/OFQ given intravesically was able to elicit an acute inhibitory effect on voiding reflex in 9 patients with NDO but not in 5 normal subjects. A RCT by the same authors including 14 NDO patients found that N/OFQ, but not placebo, increased significantly bladder capacity and reflex volume[92] and the results were replicated in a multicenter study[93]. Further investigations are required in order to establish if available selective NOP receptor agonists may become a new pharmacological way of treatment of NDO.

CANNABINOIDS

The Cannabis Sativa (marijuana) plant contains a group of biologically active substances, termed cannabinoids (CBs). The endocannabinoid system comprises the cannabinoid receptors (CB_1 and CB_2), their endogenous ("endocannabinoids") and exogenous ("exocannabinoids", such as plant-derived and synthetic cannabinoids) ligands, and related enzymes for biosynthesis and degradation, such as fatty acid amide hydrolase (FAAH)[94]. Recently, an orphan human G-protein coupled receptor, GPR55, was claimed to be a novel cannabinoid receptor[95].

These components have been located to animal and human lower urinary tract tissues (detrusor, bladder afferent nerves, and, particularly, urothelium) and have been involved in regulation of bladder function and bladder inflammation[94,96-100]. Intravesical, intrathecal and systemic administered CB-agonists are reported to inhibit bladder afferent signalling in animal models of bladder inflammation and improve urodynamics parameters in naive and DO animals models[98,101-103]. Plasticity of the endocannabinoid system in the spinal cord has been reported in rats with BOO-induced DO[104].

In patients with MS, cannabis extracts and delta-9-tetrahydrocannabinol (THC) were found to reduce OAB symptoms in open-label[105] and randomized trials[106], respectively. Nabiximols (Sativex, GW Pharmaceuticals), a standardazid mixture of compounds (mainly THC and cannabidiol) derived from Cannabis plants, failed to achieve primary endpoint (incontinence episodes) in a RCT including MS patients with OAB, however significantly improved other OAB symptoms (*e.g.*, voids per 24 h, nocturia, and bladder symptom severity)[107].

Neurological side-effects of CB_1-agonists, together with the unknown consequences of long-term use of such drugs, generated concern about their safety[108]. However, the intravesical administration of CB-agonists, the possible exploitation of CB_2 (mainly peripheral) receptors and the inhibition of the FAAH by systemic, intravesical or intratecal-administered inhibitors may be alternative approaches to target the endocannabinoid system averting CNS side-effects[94,97,104,109,110].

ANTIANDROGENS

Gonadotropin releasing hormone receptor (GnRH-R) antagonists have been reported to have beneficial effects on LUTS in patients with benign prostatic hyperplasia (BPH)[111], although they are considered still investigational in this setting, especially in light of the disappointing results of a phase Ⅲ RCT on cetrorelix[112].

Treatment with subcutaneous degarelix (Ferring), a long-acting GnRH-R antagonist, improved experimental

DO in rats and also promoted more efficient bladder emptying; isolated detrusor from degarelix-treated rats responded with larger carbachol-contractions than controls[113]. Another compound in this class, ganirelix, given systemically counteracted experimental DO in rats[114]. Interestingly, intravesical ganirelix and degarelix improved urodynamic parameters in rats[113,114]. Based on these results and since the GnRH-R is expressed in the rat bladder[113], a local intravesical administration of this class of drugs may be considered.

PHOSPHODIESTERASE INHIBITORS

Phosphodiesterases (PDE) are enzymes that degrading cyclic nucleotides (cAMP and cGMP), can counteract the detrusor relaxation[115]. Among eleven PDE isoforms so far identified, PDE1-5 are described in the bladder and preclinical studies showed that PDE inhibitors (PDE-Is) are able to reverse the cholinergic-induced contraction of human detrusor and to enhance cAMP/cGMP-mediated detrusor relaxation[116]. Selective inhibitors of the different PDE types have been showed to can counteract DO[117].

Although a pilot study suggested a possible role for vinpocetine, a PDE1-inhibitor, in the treatment of refractory OAB[118], in a multicentre, placebo-controlled RCT in patients with DO, vinpocetine showed a statistically significant superiority over placebo for only one parameter[115].

Rolipram, a PDE4-I, has been showed to inhibit phasic myogenic contractile activity of human detrusor[119]. Other PDE4-I have been showed to reduced DO in rats with BOO, without affecting the voiding phase, suggesting that PDE4-Is might represent an alternative strategy for the treatment of the OAB[120,121].

A PDE9-I (ASP4901) is also under evaluation in a phase Ⅱ trial by Astellas Pharma enrolling male patients with BPH[122].

Sildenafil, a PDE5-I, reversed the tonic cholinergic-induced contraction of human detrusor smooth muscle and produced relaxation *via* activation of cGMP- and cAMP-dependent pathways, K^+ channels and the hydrogen sulfide [H(2)S] signaling pathway[123,124]. A series of RCTs provided substantive evidence of the efficacy and tolerability of PDE5-Is (sildenafil, tadalafil, vardenafil, and United Kingdom-369003) for the treatment of LUTS in male patients with or without erectyle dysfunction, confirmed by meta-analyses[125,126]. Tadalafil received the FDA approval in October 2011 for the treatment of males with LUTS secondary to BPH or concurrent LUTS and ED.

PDE-Is require further evaluations in order to better define their mechanism and site of action in lower urinary tract, their role and optimal dosage in different group of patients and in women, long-term safety and efficacy and cost-effectiveness.

NITRIC OXIDE-DONOR DRUGS

Nitric oxide (NO) is a potent biological messenger that promotes detrusor relaxation, likely *via* the elevation of intracellular cGMP.

HCT-1026 (nitroflurbiprofen, by NicOx SA) is a NO-releasing derivative of the nonsteroidal anti-inflammatory drugs (NSAID) flurbiprofen[127]. Nitroflurbiprofen combines the anti-inflammatory activity of flurbiprofen with the smooth muscle relaxant activity of the NO moiety and promising preclinical (internal report of NicOx SA) and phase Ⅱ clinical efficacy results have been announced in the treatment of NDO patients and women with OAB, providing a rationale for phase Ⅲ trials[128,129].

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Several lines of evidence suggest an important role of prostaglandins (PGs) in the modulation of the bladder function[130]. PGF2α, PGE1, and PGE2 slowly contract isolated animal and human detrusor and a role of PGs in the maintenance of detrusor tone and in the modulation of efferent and afferent neurotransmission has been suggested[131]. Release of PGE_2, which acts *via* mainly EP receptors, is elevated in DO due to SCI[132] or to BOO[133,134]. The intravesical instillation of PGE_2 also induces DO, urgency and decreases bladder capacity in humans[135].

PGs are locally synthesized in human bladder by constitutive (COX-1) and inducible (COX-2) cyclo-oxygenase[130]. Several factors including stretch, nerve stimulation, injury, exposure to ATP and other inflammatory mediators may induce the synthesis of PGs[131].

COX-inhibitors such as NSAID were found to be able to increase bladder capacity and prolong micturition interval without affecting voiding phase in rats, and favorable clinical effects have been reported in OAB patients treated with aspirin, indometacin, flurbiprofen, ketoprofen and loxoprofen[136,137]. Other preclinical findings have also indicated COX-2-selective inhibitors as potential drugs aimed to treat OAB, also by intravesical instillation[138,139]. It seems that NSAIDs might open a novel treatment opportunity for OAB although clinical evidence of efficacy of COX-inhibitors in OAB patients remains scarce and side-effects are important issues with these drugs[140].

PROSTAGLANDIN RECEPTOR ANTAGONISTS

The use of selective antagonists of PG receptor subtypes has been explored as a possible way to treat OAB. EP_1 and EP_2 PGs receptors have been demonstrated in the mucosal bladder network where they may trigger DO by eliciting bladder afferent activity during inflammation (possibly through TRPV1) and likely through the activation of interstitial cells[141,133]. There are data showing that EP_3 receptors also participate in PGE2-induced DO[142].

Encouraging observations reported with novel EP_1

antagonist compound (*e.g.*, ONO-8539) in animal models[134] prompted their evaluation in a clinical proof-of-concept trial with disappointing results[143], thus reducing the likelihood of an oncoming introduction of EP_1 receptor antagonists in the clinical management of OAB.

DRUGS ACTING ON RHO-KINASE PATHWAY

Rho-kinase inhibitors

The Ras homologue family member A (RhoA) is a guanosine triphosphate hydrolase (GTPase) that, together with one of its downstream effectors, the type Ⅰ and type Ⅱ Rho-kinase (ROCK), have been shown to play an important role in calcium-independent pathway of smooth muscle contraction (the so-called "calcium-sensitization") both in animal and human bladder[144-147]. The upregulation of RhoA pathway has been implicated in cystopathy associated to diabetes, BOO and DO[148].

Nonclinical *in vitro* studies showed that Y-27632 and HA-1077 (fasudil), ROCK1 and ROCK 2 inhibitors, respectively, significantly blocked carbachol-induced contractions and caused concentration-dependent relaxation of human detrusor strips[146]. It has been showed in pig urinary bladder tissues that this effect involved both urothelium-dependent and independent pathways[149].

Inhibition of Rho-kinase activity with Y-27632 produced a significant suppression of DO in spontaneously hypertensive rats (SHR) that also showed significantly higher RhoA protein expression in bladder tissues[150]. Treatment with oral fasudil partly but significantly ameliorated the development of DO in a rat model of BOO[151].

ROCK inhibitors may be a new pharmacological approach to treat OAB/DO if novel bladder-selective ROCK-inhibitors will be discovered in order to overcome the hypotensive side-effects of nonselective compounds.

Vitamin D3 receptor agonists

Vitamin D3 receptor (VDR) is expressed in prostate and bladder tissues and BKL-628 (elocalcitol, BioXell), an agonist of vitamin D3-receptors, entered the pipeline for the therapy of BPH[152,153]. Elocalcitol is able to counteract the RhoA/ROCK pathway in the prostate and in both rat bladder strips and human bladder cells[154]. Elocalcitol appears to modulate bladder contractility by decreasing calcium sensitization and increasing L-type-mediated calcium entry[154,155]. The oral treatment with elocalcitol suppressed DO in two animal models of OAB and exerted strong suppressive effect on urinary bladder sensory signaling during filling in mice[156]. Encouraging results of a proof-of-concept clinical study prompted a phase IIb RCT including 308 OAB women[157]. The primary endpoint was not met but a favourable efficacy/tolerability profile and the statistically significant improvement of relevant secondary endpoints in the elocalcitol group *vs* placebo make this compound worthy of future reappraisal.

NERVE GROWTH FACTOR BLOCKADE

It has been suggested that selective inhibitors of nerve growth factor (NGF) may be a new way to treat OAB[158]. Several findings corroborate this hypothesis: urinary NGF levels decreased after successful treatment of OAB with antimuscarinics or BoNT[159,160]; NGF overexpression in the bladder and bladder afferent pathways has been reported to be involved in the emergence of hyper-excitability in bladder C-fiber sensory pathways[161]; the intrathecal administration of NGF antibodies decreased NGF levels in bladder afferent pathways and normalized bladder/urethral function in SCI rats[162].

There is nonclinical evidence that the local instillation of antisense oligonucleotides against the NGF, suppresses DO and the expression of NGF and chemokines[163]. In particular, the intravesical liposome-delivered antisense NGF-suppressing therapy could be an attractive approach for OAB, avoiding the toxicity of systemic nonspecific blockade[163].

DRUGS ACTING ON ION CHANNELS

Potassium channels openers

Potassium channel opening drugs (KCOs) cause hyperpolarization and reduction in intracellular calcium concentration, promoting detrusor relaxation[164,165]. Furthermore, these agents may inhibit overactive bladder afferent pathways or influence the release of various urothelial mediators[166].

Many types of potassium channels have been demonstrated in the detrusor smooth muscle[167]: (1) big, intermediate and small calcium-activated channels (BK or maxi-K, IK and SK, respectively); (2) voltage-dependent (K_V) channels; (3) inward-rectifying ATP-dependent channels (K_{ATP}, also known as $K_{ir}6$ channels, a subtype of the K_{ir} channels family); and (4) two-pore-domain (K_{2P}) channels (also known as "leak potassium channels").

BK channels (also called Maxi-K or slo1) have been extensively studied in animal and human detrusor smooth muscle and are arguably the most important physiologically relevant potassium channels regulating detrusor muscle cells action potential, resting membrane potential, and contractility[168-170]. Convincing data suggest that BK channels are also involved in mediating the relaxing effects of β3-ARs stimulation[171]. An important role of BK channels has been also advocated in etiopathogenesis of DO based on experimental *in vitro* observations in animal and human bladder tissues[170-173].

The role of K_V channels in normal and pathological detrusor activity remains controversial and largely unexplored[174]. A reduction in potassium currents through K_V channels has been involved in the hyperexcitability of the afferent neurons[175,176]. The structural diversity

and the variety of the K_V channels may allow for the identification of bladder-specific channels paving the way for the development of bladder-selective agent and genetic therapies for OAB[167,177].

Promising preclinical data from both *in vitro* and *in vivo* studies have been published supporting the possibility to restore normal detrusor function with openers of BK channel[168,178] and A-type K_V channel[179,180]. Although their role is still debated, interesting preclinical observations are also available on openers of K_{ATP} channel[181-184], SK channel[185,186], combined SK/IK channel[187] and putative TREK-1 K_{2P} channel[188].

Unfortunately, clinical trials with some of these drugs (*e.g.*, ELB245 and ZD0947) were disappointing because of failure to demonstrate superiority *vs* placebo for the treatment of OAB[189], or because of side-effects leading to early termination[190]. Nevertheless, there is an ongoing effort to develop new classes of more potent and selective KCOs that may lead to the development of bladder-selective agent in the future.

Sodium channels blockers

Tetrodotoxin-resistant sodium channels (NaV1.8 subtype) are expressed in primary afferent capsaicin-responsive neurons innervating the bladder and their blockade by antisense oligodeoxy-nucleotide reduced the frequent voiding evoked by acetic acid-induced irritation of the bladder[191]. Ralfinamide (NW-1029) is a sodium channel blocker that suppresses tetrodotoxin-resistant sodium currents in C-type dorsal root ganglia neurons[192]. *Via* selective inhibition of capsaicin-responsive nociceptive neurons expressing tetrodotoxin-resistant sodium channels, ralfinamide is thought to elicit anti-nociceptive effects in animal models of inflammatory and neuropathic pain, as well as beneficial effects in DO[193].

Mechanosensitive ion channels, such as degenerin family/epithelial, amiloride-sensitive, sodium channel (ENaC) and TRP channel superfamily, have been recently demonstrated to play key roles in the mechanosensory signalling of the urinary bladder[194]. Acid-sensing (voltage-insensitive) cation channels (ASIC) are a subgroup of neuronal ENaC channels highly expressed also in the urothelium and suburothelial nerve plexus[195]. An increase in intrabladder pressure or upregulation of these channels may trigger afferent signalling during bladder filling[196]. ASIC channels seem involved in nociception in various pathological conditions including human bladder inflammation[197,198]. Consequently, ENaC/ASIC ion channels may become novel targets for the pharmacological treatment of inflammatory and overactive bladder conditions[194].

CONCLUSION

The complex neurophysiological control of the micturition reflex at both central and peripheral level, and the emerging recognition of the role of the different cell types involved in bladder physiopathology, prompted the development of many lines of research mostly aimed to the discovery of new pharmacological principles using receptor ligands as starting point. However, it appears that very few candidate agents, discovered starting from ligands-like compounds have passed the proof-of-concept stage in patient-oriented studies.

The pharmacological manipulation of central micturition circuitry is supported by the growing evidence on the central origin of OAB, although side-effects limit the use of currently available neuropharmacological agents and clinical results with selective antiserotoninergic are disappointing. It emerges a growing appreciation at preclinical level for the role of purinergic receptors as new targets for the treatment of OAB. Although the first clinical data are disappointing, NK-1 antagonists have attracted the interest of several companies and proof-of-concept studies are ongoing evaluating other compounds in this pharmacological class. Proof-of-concepts data are awaited also on novel opioids receptors agonists. Based on the recent evidence on the key role of the mucosal bladder network in the regulation of bladder function, many novel pharmacological principles targeting urothelium and afferent nerve fibers are under development. Although unsatisfactory clinical results have been reported with compounds based on this strategy (PG receptor antagonists, KCOs, elocalcitol), many other investigational agents show promise such as TRPV1-antagonists, modulators of endocannabinoid system, COX-2 inhibitors, ENaC/ASIC ion channels modulators. Intravesical strategies using N/OFQ, GnRH-R antagonist and liposome-delivered targeting NGF also deserve future investigations. Another strategy that seems encouraging is based on the modulation of second messangers by using PDE and ROCK inhibitors, and NO-donor drugs. Although the exciting expectations rose from gene therapy still need to be realized, the advances in this field are promising also in the clinical setting of OAB.

It is likely that the future will provides the clinicians with a variety of drugs, with distinctive mechanism of actions, to be used in combination or sequentially, and in groups of patients with different clinical phenotypes.

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Effect of the maternal-fetal interface immunoregulation on the occurrence of intrahepatic cholestasis of pregnancy

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Abstract

Maternal immune tolerance of the fetus is indispensable for a healthy pregnancy. Currently, the study of the immune microenvironment of the maternal-fetal interface has been a heated topic in reproductive immunology research. More and more studies show that the immune imbalance in the maternal-fetal interface plays a very important role in the incidence of intrahepatic cholestasis of pregnancy (ICP). However, the precise etiology and mechanism of immune imbalance in the occurrence of ICP is still unknown. In order to clarify the potential immunologic mechanisms of ICP, this review summarizes the recent studies of the decidual immunology micro-environment and the potential immunologic mechanisms related to the development of ICP.

Key words: Intrahepatic cholestasis of pregnancy; Decidual lymphocytes; Trophoblast; Human lymphocyte antigen-G

Core tip: In this paper, we reviewed the recent publications regarding the role of immunological interactions at the maternal-fetal interface on the occurrence of intrahepatic cholestasis. The literature shows that the decidual immunological microenvironment may relate to the development of intrahepatic cholestasis of pregnancy. Any approach that modulates immune tolerance at the maternal-fetal interface toward the natural state could provide insight in the treatment of intrahepatic cholestasis of pregnancy.

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a metabolic disease occurring in the second and third trimester. Experiments show that bile acids have

cytotoxic effects, leading to apoptosis and necrosis *in vivo*. Exposure to high blood bile acids in pregnancy can cause damage to the fetal heart, liver, lungs and other organs[1]. In addition, bile acids can increase the expression of oxytocin receptors in the uterine muscle fibers increasing sensitivity to oxytocin[2]. These factors can lead to vasospasm and hypoxia at the surface of the placenta and increased vascular permeability[3]. Thus ICP is an important risk factor for perinatal morbidity and mortality[4]. The etiology and pathogenesis of ICP is not clear. It may be related to the mutation of genes such as *MDR3*, *BSEP*, *ATP8B1*[5-7], imbalance of estrogen and progesterone[8], or thyroid hormones[9]. These changes may cause metabolism disorders. When this happens in the liver, the Na^+-K^+-ATP activity and the biliary bile salt transportation function may decrease, inducing intrahepatic cholestasis. Over the past decade, the relationship of placental immunological changes has been found to be increasingly related to the development of ICP[10]. The embryo, as an alloantigen with paternal ingredients, is essentially an allogeneic allograft to the uterus. The maternal immune system identifies the fetus as an allograft, gradually establishing a balance of tolerance between the maternal and fetal components of the pregnancy.

Once this balance is disrupted, the maternal-fetal interface's unique dynamics in the local immune microenvironment cannot be maintained, resulting in direct placental trophoblast and decidual cell damage, while a number of inflammatory cytokines are released into the peripheral blood, resulting in changes of the immune microenvironment in liver tissue, inducing cholestasis.

Maternal-fetal immune regulation is a core issue of reproductive immunology. How the maternal-fetal immune interaction influences ICP has plagued scientists. Maternal-fetal interface immune regulation during pregnancy in ICP has been a hot research topic in recent years. A systemic review on the correlation between the etiology and pathology of the maternal fetal interface immunity will help to clarify the immunological mechanisms of ICP and promote and improve the diagnosis, treatment, and understanding of ICP.

RESEARCH ADVANCES ON IMMUNE TOLERANCE BETWEEN THE MATERNAL-FETAL INTERFACE

The maternal-fetal interface is a direct maternal and fetal tissue contact surface, which consists of a large number of immune cells, decidual cells and fetal trophoblast cells. Nevertheless, the number of immune cells, surface antigens, and the effect on receptors in the maternal-fetal interface are very different compared to other organs. Such immune conditions in the maternal-fetal interface protect the placenta as an immune privileged organ. This kind of immune tolerance occurs in the interaction between decidual cells and trophoblast cells.

In general, the immune tolerance embodied in the maternal-fetal interface consists of cellular and humoral immune tolerance. Maternal decidual immune cells include NK cells and T lymphocytes. Studies demonstrate that more than 80% of the maternal-fetal interface lymphocytes are NK cells and the decidual NK cells are mainly CD^{56+}, which secrete cytokines and growth factors to induce local immunosuppression and help embryo development[11]. It is reported that the type of T lymphocytes in pregnancy also shows a decreased CD^{4+}/CD^{8+} ratio, as well as a helper T cell (Th)1-Th2 shift phenomenon[12]. Th1 secrete interleukin (IL)-2, IL-12, interferon (IFN)-γ and tumor necrosis factor (TNF)-β/α mediated cytotoxic response; Th2 secrete IL-4, IL-5, IL-6 and IL-10, inducing a humoral immune response, and stimulation of B lymphocytes produces antibodies[13]. Studies have shown a shift in the balance of cytokine profiles away from Th1 type reaction to Th2 type reaction in ICP. It disrupts the immune tolerance balance between mother and fetus[10,14]. Th3 secrete transforming growth factor β1 (TGF-β1) which has a strong immunosuppressive effect on cytotoxic T cells, natural killer cells, T cells and natural killer T cells[15]. It is well established that the fetal trophoblast does not express classical human lymphocyte antigen (HLA)-A and HLA-B which can be identified by maternal decidual T lymphocytes, but has a high expression of non-classical HLA-G and HLA-E, and HLA-I like proteins[16,17]. Thus, damage to the fetus from NK cells and T lymphocytes of the mother is prevented.

Kovats *et al*[18] found that maternal expression of leukemia inhibitory factor (LIF) begins early in pregnancy. LIF can selectively induce HLA-G expression in the maternal-fetal interface on the outer membrane of the chorionic trophoblast cells[18]. $CD8^+$ and $CD4^+$ T cells interact with HLA-G in trophoblast cells to activate the Fas/Fasl signaling pathway, inducing $CD8^+$ T cell apoptosis, and inhibiting the proliferation of $CD4^+$ T-positive cells. This may be the reason why T cells in the decidua are far less numerous than in the outer periphery circulation[19]. Bainbridge proved that the leading strand of HLA-G can strongly induce the expression of HLA-E in the outer chorionic trophoblasts[20]. And the decidual NK cells expression of CD94/NKG2A receptors is 5 fold that of the periphery circulation[21]. Carretero found that the CD94/NKG2A receptor and HLA-E binding by SHP-1 molecules recruits the intracellular delivery of immune inhibitory signals. At the same time, it is found that the HLA-G is the ligand for the expression of the KIR 2DL4 and ILT2 receptors in NK cells in the decidual membrane. Their interaction could inhibit the activity of NK cells[19]. Thus, trophoblast cells are immune to injury by the expression of non-MHC-I receptors to inhibit antigens from NK cells. In addition, HLA-G and HLA-E could inhibit the killing effect of macrophages and T cells on the decidual membrane[22].

Humoral immune tolerance at the maternal fetal interface is effected through blocking antibody (BA) IgG and its subclasses. BA are produced idiotypically of anti-anti-HLA antibodies which are reactive with the fetal half of the self HLS antigens[23]. It can bind the epitope surface antigen of the trophoblast to avoid the maternal killer T cell recognition, allowing the fetus to escape the maternal immune response[24]. Studies show that the HLA-G antigen can stimulate maternal BA production. Furthermore, placental BA concentration is 3 fold that of the maternal symmetric antibody concentration. Th2 factor IL-6 can regulate the production of blocking antibodies through glycosylation[25].

IMMUNE TOLERANCE DISORDER AND THE PATHOGENESIS OF ICP

In recent years, the study of immune pathogenesis in ICP has become a popular research area. More and more research shows that the dynamic imbalance of the maternal fetal interface is closely related to immune tolerance in ICP[26]. Patients with ICP usually demonstrate changes in humoral and cellular immune tolerance associated with antigens, receptors and cytokines, and these changes are not only reflected in the maternal fetal interface but also in maternal peripheral blood.

Studies have shown that the level of IgG in the serum of pregnant women with ICP is significantly decreased, but IgM, IgA and C3, C4 show no significant changes. This suggests that decreased IgG blocking antibody leads to a weakened immune protection effect and failure of maternal fetal immune tolerance.

Cellular immune microenvironment changes in ICP

Studies have shown that TNF-α concentration is higher in peripheral blood of women with ICP than in pregnant women without ICP. It is also confirmed that a decreased TGF-β1 in ICP placental tissue can promote secretion of TNFα and IL-1. The expression of TNF-α and IL-1 are increased in placental tissue in patients with ICP, which promote the secretion of TGF-1[27]. Recent studies have found that TNF-α increase significantly in serum of ICP patients. The increase of TNF-α is positively related to the severity of ICP, which demonstrates that TNF-α may be involved in the occurrence of ICP[28]. Prior to these findings, it was found that an increasing IFN-γ expression in placental tissue from ICP patients as well as TNF-α was related to the occurrence of ICP[29,30]. Increases of IFN-γ and IL-4 may play an important role in the pathogenesis of ICP[31]. These results suggest that ICP generally shows an increased Th1 type cytokine phenomenon, leading to a increase of the Th1-Th2 ratio demonstrating pathological changes of cell immune imbalance[32].

Immune cellular surface antigen changes in ICP

Changes of Th2-Th1 cells in the maternal fetal interface are likely to represent the cellular immune reaction activity of the maternal-fetal interface. The changes of the cellular immune surface antigen of the maternal fetal interface in ICP have received much research attention. A decrease in $CD8^+$ cells and an increase in $CD4^+$ cells in ICP patients lead to an increase of CD4/CD8 ratio. Meanwhile, it is also found that NK cells decrease significantly[14,33]. In recent studies, it is found that leukocyte CD3 antigen, CD4/CD8 ratio, and Th1 were all elevated in the ICP group compared to the control group, while CD8 and Th2 were lower in the ICP group than in the control group, Women with ICP have abnormal expression of T cells and helper T cells in peripheral blood[26,34].

The results from these studies demonstrate that the surface antigen on Th2 cells and NK cells have enhanced cellular immune function, and promote the Th1/Th2 type cytokine balance *via* Th1. The lack of such balance may be a cause of the liver cell damage noted to be present in ICP.

Trophoblast cell surface antigen and decidual cell receptor changes in ICP

Immune tolerance in pregnancy begins as immune recognition of decidual cells and trophoblast cells. High expression of specific inhibitory ligands on trophoblast cells and specific inhibitory receptors of NK cells in decidua are important factors of immune tolerance. Changes in the expression of trophoblast cell surface antigens and decidual cell receptors inevitably lead to immune dysfunction, which may cause ICP. Peng *et al*[21] found that decreased expression of HLA-G and HLA-E protein in trophoblast cells are related to ICP. It may be one of the important mechanisms involved in pregnancy immune tolerance imbalance disorders[21]. Dexamethasone increases expression of HLA-G and HLA-E, which may be the critical pharmacological mechanism in the treatment of ICP. Other studies found increased Th1 and decreased HLA-2G in placental tissues from ICP patients, which demonstrate that changes of surface antigens on trophoblast cells may be another cause of ICP[29]. In a different study, downward expression of VEGF receptors was found in syncytiotrophoblast, cytotrophoblast, placental vascular endothelial cells and villi in ICP placentas but it is not clear which is the cause and which is the effect[35].

IMMUNE ETIOLOGY STUDIES ON ICP

Genetic factors influence immune balance of ICP

Genetic factors in the pathogenesis of ICP have been studied for several decades. Many studies have shown that ICP may be the result of multiple factors including a well recognized genetic predispositions[36-39]. That some liver cell secretory transport genes act as genetic factors in the pathogenesis of ICP has been confirmed, but the relationship between immunologically related genes and ICP is not clear. Some scholars believe that the HLA-Ⅱ antigen promotes the production of blocking antibody. The compatibility is high when the mother and fetus have similar HLA-Ⅱ antigen expression. The

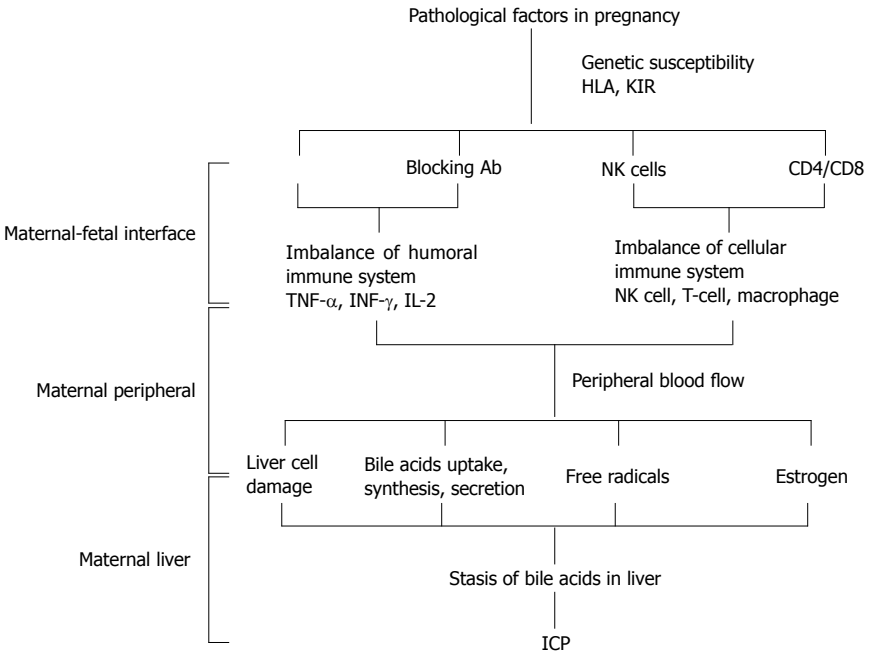

Figure 1 Pathogenesis of intrahepatic cholestasis of pregnancy caused by immune intolerance. ICP: Intrahepatic cholestasis of pregnancy; KIR: Killer cell immunoglobulin-like receptor; IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor; HLA: Human lymphocyte antigen.

higher the compatibility of the maternal fetal HLA-Ⅱ antigen, the less the immune response. The strength of the maternal immune system response to paternal antigens of the fetus correlates to the susceptibility of the onset of ICP[40]. Nowak pointed out that women with activation of killer cell immunoglobulin-like receptor (*KIR*) gene and the KIR inhibitory receptor gene ratio between 0.33-0.83 were prone to have spontaneous abortion while women with a ratio between 0.86-1.25 tend to have a NK cell protective effect, suggesting that the KIR genotypes most likely associate with pathologic pregnancies, such as spontaneous abortion[30,41]. Guimond found that women with missing T cells may have a normal pregnancy but those missing NK cells exhibit pathological changes of pregnancy. He believes these results demonstrate that NK cell immune activity not only has potential immune protective effects, but also is the main cellular immune recognition mediated by the maternal fetal interface[31,42]. These prompted explanation of immune genetic factors in the pathogenesis of ICP, including the ligand NK cell surface antigen, receptor, and their interaction in trophoblast cells. Unfortunately the genetic mechanism for the pathogenesis of ICP in immune gene is still not clear.

Pathogenesis factors disrupt immune tolerance during pregnancy

Without a doubt, research results have clarified our understanding of the changes in immune cell activation and other immune factors in ICP. But factors disrupting the balance of immune tolerance in the maternal fetal interface is not clear. The study of immune tolerance disorders has received little attention. Excluding genetic susceptibility factors, it is not difficult to understand why some factors which cause strong immune responses likely to be associated with ICP. In addition, maternal factors such as nerve - endocrine interactions also affect the decidual immune microenvironment. Social and psychological stress may cause an increase of proinflammatory cytokines. Stress during pregnancy causes changes of IL-6 and IL-10 levels[43,44]. Even though IL-6 and IL-10 level are associated with ICP[45], further studies are necessary to clarify whether prenatal stress may be part of the pathogenesis of ICP.

TO INVESTIGATE THE IMMUNE IMBALANCE AS A MECHANISM OF ICP

Is immune dysfunction the cause of ICP or the result? If the immune imbalance is the result of ICP, then what is the pathogenesis? Previous studies of immunologic changes in ICP seem unable to determine immune dysfunction and ICP causality. It is difficult to carry out research on the causality of ICP because changes in immune regulation cannot be monitored before the onset of the maternal-fetal interface to clarify the immune changes with the onset of ICP. However, we still have some evidence to support that immune regulation disorders can cause the occurrence of ICP. Peng found that dexamethasone can treat ICP and up-regulate HLA-G and HLA-E, which suggests that the expression of surface antigen on trophoblast in ICP can be effectively influenced. This supports the notion that immune imbalance is more likely the initiating factor of ICP[46].

In addition, the up-regulation of Th1 type cytokines such as IL-2, IL-12, IFN-γ, and TNF-α/β and their

release into the maternal circulation can generate liver injury and induce ICP. Interaction of TNF-α and IL-2 can promote NK cell change into cytotoxic LAK cells, resulting in damage to liver cells. TNF can also activate neutrophils, promote their aggregation in the liver and prompt degranulation, releasing proteases and oxygen free radicals, causing liver cell damage[27,47]. In addition, TNF-α can promote the uptake, synthesis, and secretion of bile acids in liver cells resulting in cholestasis[45,48]. Furthermore, TNF-α increases the synthesis and secretion of placental estrogen, which is associated with ICP[49].

In conclusion, immune pathogenesis leading to ICP might including the following: In the genetically susceptible population, pathogenic factors promote changes in gene expression of trophoblast leukocyte antigen and receptors on decidual NK cells, which lead to an unbalance of blocking antibody and cytokine pathways. Then the cellular immune system activates and secretes Th1 cytokines into the maternal circulation causing damage to the liver cells, resulting in ICP (Figure 1).

Thus, these pathological changes contribute to an increased fetal morbidity due to maternal changes, mediated (at least in part) by disruption of the maternal-fetal immune balance. Because of the etiological factor and the pathogenesis of ICP, there is currently no effective clinical standard to prevent and cure ICP. Any approach that modulates the immune tolerance of the maternal-fetal interface toward the natural state could provide insight in the understanding of ICP, which could lead to a targeted treatment.

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MINIREVIEWS

Mental health of perinatal women

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Abstract

Pregnancy and childbirth are major stressors for some women. They can be followed by deterioration in mental health status and cause mental illnesses during perinatal period. Undetected and untreated perinatal mental illnesses can have negative unexpected impacts on parenting skills of the women and children's development. Mentally ill mothers may not effectively attend their children's needs in a timely manner and may experience an unfavourable mother-child attachment affecting the child's language, social, emotional and cognitive development. The rate of pregnancy and postnatal health complications and interventions is higher among mentally ill women with some certain risk factors. The mentally ill mothers along with their partners need comprehensive support and counselling to be able to care for their infants and establish strong parent-child bond and attachment. Mental health campaigns across the world have endeavoured to increase the knowledge and awareness of the public towards perinatal mental health illnesses. To this aim, a routine screening is recommended in order to identify the women who are at risk of mood or anxiety disorder during perinatal period. The development of knowledge on perinatal mental illnesses among public and the health professionals has resulted in timely recognition and treatment of perinatal mental illnesses. Although great volumes of research show high prevalence of perinatal mental illnesses and their impacts on parenting confidence and competence as well as child's developmental process, there is still lack of research on various aspects of perinatal mental illnesses. To enable early prevention, diagnosis and intervention, it is crucial to identify families who are at an increased risk of perinatal mental illnesses and provide support and intervention to minimise the adverse outcomes. The children's needs may not be met by providing treatment to parental mental illnesses alone. It is also important to understand the impact of specific parenting behaviours on child outcomes which is modified by the quality of parenting.

Key words: Perinatal mental illness; Depression; Anxiety; Pregnancy; Childbirth

Core tip: Pregnancy and childbirth are major stressors for some women. Undetected and untreated perinatal mental illnesses can have negative unexpected impacts on parenting skills of the women and children's development. Mentally ill mothers may experience an unfavourable mother-child attachment. Perinatal mental illness affects the child's language, social, emotional and cognitive development.

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INTRODUCTION

Pregnancy and childbirth are expected to be blessing times in women's life, when physiological and psychological changes prepare the women for motherhood tasks. Pregnancy and childbirth are, however, perceived as major stressors for some women as they struggle with self-depreciation and undermining of self-esteem followed by feeling incapable of caring for the newborn. These negative feelings are followed by deterioration in mental health status and can cause mental illnesses during perinatal period[1].

Perinatal mental illnesses are described as "psychiatric disorders that are prevalent during pregnancy and as long as 1 year after delivery"[2]. Various types of perinatal mental illnesses have been reported in the literature including postpartum blues, perinatal depression, post-partum anxiety disorders and postpartum psychosis (bipolar disorders)[2].

Perinatal mental illness has been identified as one of the most important issues in women's health. Perinatal mental illnesses negatively affect women's interpersonal relationship and quality of life and have direct and indirect negative impacts on short-term and long-term physical and mental health of their children. The greater concern is for those women who abstain to disclose their mental health problems due to fears of stigma, losing parental rights and being judged as incompetent and unqualified parents. Some women also discontinue their psychiatric medications during pregnancy and lactation due to concerns regarding the baby's well-being[3], which can result in an increased risk of suicidal thoughts or attempts at self harm[4-6].

PREVALENCE AND RISK FACTORS OF PERINATAL MENTAL ILLNESSES

A review of the literature shows that although any woman can experience perinatal mental illness, this problem is not randomly distributed among the population of perinatal women. A combination of biological, socio-environmental and psychological factors can affect mental health of women across their life span and predict their mental illnesses[7] (Table 1). A previous history of mental health problem, such as depression or anxiety, has been reported to be a strong predictor of perinatal mental illnesses. Half the women with prenatal depression will continue to feel depressed throughout pregnancy and during postnatal period. Major depressive disorder and bipolar episodes may also occur before pregnancy and relapse during pregnancy and after chidlbirth[1]. Nevertheless, somatic complaints and sleep difficulties may be attributed to the changes happening during pregnancy and postnatal, obscuring the diagnosis of the mental illnesses and leaving the women with no appropriate treatment[8].

According to the report by the World Health Organisation, the mean prevalence of non-psychotic common mental disorders in low- and lower-middle-income countries was 15.6% during antenatal period and 19.8% postnatal. Factors such as a higher education, a permanent job, the ethnic majority and having a supportive intimate partner were shown to be protective against mental health problems[9].

Research has shown that women who carry the following risk factors are more likely to develop mental illnesses during pregnancy and after childbirth: socioeconomic disadvantage, a history of trauma, sexual abuse, unplanned pregnancy, high risk pregnancy, young age, being unmarried, lack of support from the intimate partner, intimate partner violence, inter-personal issues with in-laws, insufficient emotional and practical support, giving birth to a female baby, low level of education, cigarette smoking, career insecurity and ethnic minority[2,9-11].

The reports from the Beyondblue postnatal depression screening program show that 5%-10% of Australian women experienced symptoms of depression after childbirth[12]. A population-based survey by Eastwood *et al*[13] demonstrated that the prevalence of postnatal depression after 2 wk postpartum was 6.2%. It was also reported that the risk of postnatal depression was significantly associated with maternal country of birth, financial difficulties, unplanned pregnancy, not breastfeeding and poor maternal health.

The study by Melville *et al*[14] in the United States showed that the prevalence of antenatal depressive disorders was 9.9% and panic disorder was 3.2%. In addition, 2.6% of the participants reported current suicidal thoughts. The odds of probable antepartum major depressive disorder increased in the women who reported psychosocial stress, domestic violence, chronic medical conditions and Asian and African-American ethnic group.

PERINATAL MENTAL ILLNESS AND CHILD'S WELL-BEING

Women with mental illnesses are less likely to care for themselves during pregnancy and after childbirth. Research has shown that maternal anxiety during pregnancy is associated with higher level of cortisol in the fetus which continues to be higher than normal levels throughout the child's life span and may be a marker for the children's anxiety, mood and behavioural disorders. The risk is even higher in women who continue to suffer mental illness from pregnancy to postnatal period[15].

Table 1 Risk factors of perinatal mental illnesses

Socioeconomic disadvantage	High risk pregnancy
Young age	Giving birth to a female baby
Maternal country of birth	Being unmarried
Ethnic minority	Lack of support from the intimate partner
Low level of education	Intimate partner violence
Financial difficulties	Inter-personal issues with in-laws
Career insecurity	Insufficient emotional and practical support
Cigarette smoking	Not breastfeeding
History of trauma	Previous history of mental health problem
Sexual abuse	
Unplanned pregnancy	

The association between antenatal depression and fetal and neonatal outcomes have been investigated in two meta-analyses[16,17]. Reports of the studies indicate a significant association between antenatal depression and an increased risk of premature delivery (less than 37 wk of gestation) and infant's low birth weight (especially when mother lives in a low-income country). It was, also, suggested that the level of risk depends on the severity of the symptoms of depression.

The rate of pregnancy and postnatal health complications and interventions is higher among women with perinatal mental illness and their infants require higher rate of intensive care[18,19]. It has been shown that children of these mothers are at higher risk of child neglect, maltreatment, attachment difficulties, delayed growth and motor development, emotional problems and a range of negative cognitive outcomes in early childhood. Nutritional neglect, severe starving and malnutrition are also other enormous problems in these children most of whom are females and below five years of age[20-22]. In addition these children are at heightened risk of clinical depression in late adolescence while suffering the consequences of stress associated with caring for their mentally ill parent/s. These issues have resulted in increased concerns regarding the child's wellbeing in the family and have brought a great numbers of families into the attention of child protection agencies[23-26].

PREVENTION AND DIAGNOSIS

Early detection of any mental health problems can help prevent future serious psychological disorders. Not only women with symptoms of depression need assessment and evaluation of psychological problems, but also all well women need to be screened as part of their perinatal health check. The American College of Obstetricians and Gynecologists (ACOG) states that "screening for depression has the potential to benefit a woman and her family and should be strongly considered. Women with a positive assessment require follow-up evaluation and treatment if indicated"[27].

Child and Family Health nurses, general practitioners and obstetricians, as the primary care providers, are the first and most often point of contact for perinatal women. This provides them with a great opportunity to effectively detect perinatal psychological problems and identify those who need care and support. There are, however, some barriers to timely screening and identification of the perinatal mental illnesses by the obstetricians. Research has shown that some obstetricians feel unconfident in their own level of knowledge and believe that they have had inadequate training to provide mental health support and assistance. Time constraint during each visit has also been reported as another barrier to effective screening[28].

Pediatricians can also play a significant role in screening for postnatal depression. They can provide support to the mothers and facilitate their access to appropriate professional resources. This can in turn help optimise the healthy development of the children followed by the healthy functioning of the entire family. Similar to other screening initiatives, there are barriers to implementation of this practice such as lack of time, inadequate training, lack of sufficient mental health referral resources and reimbursement insecurity[29,30].

Research by Kim *et al*[31] reported barriers to mental health diagnosis and treatment at four levels as follows: Patient level (including lack of time, using other support and spontaneous improvement of symptoms); Provider level (including provider unavailability and unresponsive provider); Patient-provider interaction level (including poor match to need, poor patient-provider fit and use of phone tag); System level (including cost-insurance mismatch, geographic mismatch and inconvenient location).

TREATMENT

The mentally ill mothers need comprehensive support and counselling to be able to care for their infants and establish strong mother-child bond and attachment[32,33]. Not only mothers but also fathers need psychological interventions during perinatal period as they may be well affected by the changes during pregnancy and after childbirth and are prone to mental illnesses. Even when the fathers become involved in the assessment and treatment, the majority of the therapeutic options focus on the treatment of maternal or paternal mental illnesses in isolation. Since the wellbeing of both parents is important in achieving normal development of the child, there is a need for inclusive perinatal mental health care. The wellbeing of both parents should be taken into account simultaneously and the fathers need to be routinely involved in the mental health assessment and care plan, which in turn improves the health outcomes for the whole family[34,35].

MENTAL HEALTH INITIATIVES

Mental health campaigns across the world have

endeavoured to increase the knowledge and awareness of the public towards perinatal mental health illnesses. The development of knowledge on perinatal mental illnesses among public and the health professionals has resulted in timely recognition and treatment of perinatal mental illnesses. During the perinatal period, GPs and Child and Family Health nurses are key primary care providers engaged with women. They can identify women at risk, offer them an effective pathway and help alleviate postnatal mental health problems for the majority of women[36,37].

Research on mental health problems in the United States and other countries have demonstrated that despite high prevalence rates of mental illnesses in these countries, many people do not seek professional advice and support or delay seeking help for as long as they can. For instance, the World Health Initiative by the World Health Organization[38] investigated data from 28 countries and showed that both in developed and developing countries only a small proportion of the population received treatment for their mental illnesses such as mood or anxiety disorders. It was also shown that the median delays to receive treatment for severe psychotic disorders was a few months, for mood disorders ranged from one to 14 years and for anxiety disorders ranged from three to 30 years.

In a telephone survey, Highet *et al*[39] recruited 1201 adults from each State and Territory of Australia in 2009. Results of the study demonstrated that 43.6% of the participants believed that postnatal depression was the most common health problem for women after childbirth. Furthermore, 94% of the participants believed that postnatal depression needs timely and specialised treatment. About two-third of the participants perceived postnatal depression as a biological rather than psychosocial aetiology. It was also revealed that more than 80% of the adult Australians believed that there should be a routine assessment for depression in all new mothers. Nevertheless, 55% of them viewed antenatal depression as a "normal" condition.

Results of a more recent survey in 2012 demonstrated remarkable improvements in mental health literacy in the Australian population over 16 years including improved recognition of depression, better perception about the usefulness of health professionals (General Practitioners, psychiatrists, psychologists and mental health nurses) and increase in beliefs about the helpfulness of antidepressants and antipsychotics[40].

Failure to receive treatment or delay in seeking support and advice can have serious consequences. Therefore, early detection and prevention of mental illnesses is crucial and can be achieved by establishing a routine screening during perinatal period. It has been recommended that a comprehensive primary health care assessment as well as an enquiry of a history of anxiety, depression or other mental health problems are conducted at the following times: (1) Antenatally: At the first appointment with the clinician for antenatal care before 20 wk of pregnancy; (2) Postnatally: At the first postnatal home visit by the clinician; (3) Six to eight week postnatal check: Performed by the child and family health service; and (4) A further assessment at 6-8 mo postpartum. It is also recommended that the Edinburgh Postnatal Depression Scale should be administered at each visit during antenatal and postnatal period[41].

CONCLUSION AND DIRECTION FOR FUTURE RESEARCH

Undetected and untreated perinatal mental illnesses can have many negative unexpected impacts on parenting skills of women and children's development. Mentally ill mothers may not effectively attend their children's needs in a timely manner and may experience an unfavourable mother-child attachment affecting the child's language, social, emotional and cognitive development. It is crucial to identify families who are at an increased risk of perinatal mental illnesses in order to enable early prevention, diagnosis and intervention and prevent the serious adverse outcomes. The children's needs may not, however, be met by providing treatment to parental mental illnesses alone. It is important to understand the impact of specific parenting behaviours on child outcomes which is modified by the quality of parenting[42].

Although great volumes of research show high prevalence of perinatal mental illnesses and their impacts on parenting confidence and competence as well as child's developmental process, there is still lack of research on various aspects of perinatal mental illnesses. There is a need for further research to investigate parenting education interventions for parents with perinatal mental disorders. No clinical trial has investigated the effect of psychological interventions and counselling on parenting skills or child's outcomes to find out whether children benefit from the interventions. Future longitudinal, population-based studies may unearth how environmental factors, such as education and social support, moderate the influence of perinatal mental illnesses on the child outcomes, which may help to find preventive approaches. In addition, the focus of most interventional trials has been on postnatal mental illness, but not the delivery of the intervention prophylactically before or during pregnancy and its long-term impacts on the mental health of the whole family[2]. It is hoped that future studies and their findings will assist families affected by these problems.

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EDITORIAL

Urgent need to change clinical practices about postpartum contraception

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Abstract

In the United States, maternal mortality and unintended pregnancy rates are increasing. There are growing disparities in maternal health between indigent, minority women and Caucasian women of higher socioeconomic status. Family planning has long been viewed as a solution to these problems. As reliance on permanent contraception has diminished, timely access to highly effective contraceptive methods, namely long acting reversible contraceptives, which includes the contraceptive hormonal implant and intrauterine device - has become even more important. For women in the United States and abroad, the time of delivery is the one reliable opportunity for women to receive medical care. Consistently, research has shown that providing contraception in the immediate postpartum period is safe, effective, feasible and cost effective. However, misperceptions, lack of supplies, and reimbursement issues combine to defeat attempts to provide the most effective methods of contraception during that hospitalization. We believe that it is time to tackle the problem of unintended and rapid repeat pregnancy using an evidence-based, patient-centered paradigm and to eradicate systemic barriers blocking access to contraceptive methods during hospital stay. This editorial will outline some of the more compelling evidence supporting this move and will provide insights from successful programs.

Key words: Postpartum contraception; Long acting reversible contraception; Subdermal contraceptive implant; Intrauterine device; Unintended pregnancy

Core tip: The postpartum period is an ideal opportunity to initiate highly effective contraception, yet many women leave the hospital without any contraception. Provision of highly effective contraceptives, in parti-

cular long acting reversible contraceptives, such as intrauterine devices and contraceptive implants, is safe, desired, effective and cost saving. We review the need for immediate postpartum contraception and recommend changes within the medical system to facilitate this change.

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INTRODUCTION

For the last three decades, the overall unintended pregnancy rate in the United States has been nearly constant at 50%. The 2014 estimate demonstrates that the overall rate has increased to 51%, but that greater disparities exist today than ever before. The rate of unintended pregnancy has declined to 34% among women of higher socioeconomic status (SES), but has increased to 62% among women of lower SES[1,2]. Unintended pregnancies can have severe health and economic consequences for both the mother and her fetus[3]. This is perhaps most notable among women with rapid repeat pregnancies (defined as a pregnancy 12-18 mo after delivery), which is linked to increased maternal and child morbidity and mortality[4-9]. Pregnancy rates within one year of delivery range from 6%-40% depending on the population studied, and are particularly high among adolescents[10-12].

The provision of highly effective contraception in the postpartum period serves as a partial solution to the problem of unintended pregnancy and is uniquely able to drastically reduce rapid repeat pregnancy rates. Traditionally, the only contraception offered in the immediate postpartum period has been tubal ligation or progestin only pills. This is because it has been assumed that couples will remain abstinent for at least 6 wk, as instructed by the obstetrician. The more highly effective, reversible methods of contraception typically are not offered until the six week postpartum visit. The timing of this postpartum visit itself is anachronistic; it was designed to ensure the cervix and vagina had normalized so that the women could have a pap smear and a diaphragm fitting[13]. Unfortunately, clinging to this outdated standard creates barriers to accessing effective contraceptive methods and increases the risk for unintended pregnancy for several reasons. First, up to 35% of postpartum women (often the most vulnerable ones) do not return for postpartum care[11,12]. This is due often to changing insurance status. (In the United States, prenatal care, delivery and postpartum care up to 6 wk post-delivery are covered universally for low income citizens. For undocumented residents, however, only delivery care is covered generally). Among those who do present, other barriers are often encountered, such as need to order long acting reversible contraceptives (LARC) devices (which necessitates yet another visit for placement) and lack of enthusiasm on the part of physicians. Surveys of practicing obstetrician/gynecologists and family physicians consistently show a lack of knowledge of LARC, specifically regarding intrauterine device (IUD) placement[14-17]. It is not surprising that only a fraction those desiring LARC actually receive LARC at this visit[11,18]. Indeed, Potter found that while 25% of women desired LARC postpartum, only 12% actually were able to initiate LARC within 6 mo of delivery[18]. This disconnect mostly affects women of lower socioeconomic status and women who lost insurance coverage. Finally, even if women manage to present for postpartum care and are offered effective contraceptives, the visit may be too late for some women - as ovulation may return as early as 25 d postpartum for nonbreastfeeding women[19] and many couples do not observe the recommended 6 wk of abstinence postpartum[20,21].

Provision of contraception in the immediate postpartum period has the potential to solve these problems. We hope to generate greater support for this practice by demonstrating the safety, effectiveness, patient satisfaction, and cost effectiveness of immediate initiation of top tier contraceptive methods.

IMMEDIATE POSTPARTUM CONTRACEPTION IS SAFE

The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have provided clear guidance regarding the safety of immediate postpartum contraception. All progestin only methods are Category 1 (no contraindication) in nonbreastfeeding women and Category 2 (benefits outweigh risk) in breastfeeding women. Only combination methods containing estrogen are unsafe for at least 21 d postpartum due to associated risk for venous thromboembolic event (VTE)[22-24]. For women with known risk factors for VTE, initiation of combination hormonal methods should be delayed even longer until 42 d.

Frequently, breastfeeding women have been denied immediate postpartum hormonal contraceptive methods due to concerns about decreasing milk supply and/or passage of hormone into the breast-milk. Multiple studies have demonstrated that progestin only methods, including Depot Medroxyprogesterone Acetate (DMPA) and the etonogestrel implant do not delay lactogenesis[25], impede milk production[26] or adversely impact overall breastfeeding continuation rates and success[20,27,28]. Moreover, infant growth and development is not affected by hormonal contraceptives, even when provided in the immediate postpartum period[25]. The levonorgestrel (LNG) IUD is less well studied. One small study suggests that

breastfeeding rates are lower among women receiving immediate postplacental LNG IUD compared to delayed insertion, though this was a secondary analysis of data designed to evaluate IUD continuation rates[12]. There are not data regarding infant growth and development with use of a LNG IUD placed immediately postplacental. However, given that the systemic dose of progestin is significantly lower with either DMPA or the entonogestrel implant, there is little basis for concern about any adverse impact the LNG IUD could have on infant growth and development.

The placement of intrauterine devices in the immediate postpartum period (within 10 min of placental expulsion) has been shown to be safe by many metrics. Immediate postplacental IUD placement has been researched in multiple settings internationally and with multiple types of IUDs, including Lippes Loops, Delta T, Delta Loop, Gyne T, CuT380A and LNG IUD[29-39]. These studies consistently show there is no increased risk of infection with immediate postplacental placement, though women diagnosed with chorioamnionitis, chlamydia or gonorrhea in pregnancy without evidence of a negative test of cure, or ruptured membranes for more than 24 h are not candidates for immediate postplacental IUD due to infection risk[23,34,37]. No increase in perforation rates has been reported when compared to interval insertion at 6-8 wk postdelivery[37]. Postpartum pain and bleeding also do not differ when comparing women receiving immediate postplacental IUDs and women receiving no contraceptive method[34].

The risk of expulsion with postplacental IUD insertion is higher than seen with interval insertion at 6-8 wk postdelivery. The reported expulsion rate varies significantly in the literature, ranging from to 0.3% to 24%[30,32-39]. This increased risk of expulsion appears to depend on mode of delivery and interval between placental delivery and IUD placement. Studies of IUDs placed immediately after a vaginal delivery show expulsion rates of 20%-24%[31,34,36]. When the IUD is placed at the time of a cesarean section, expulsion rates are typically lower (0.3%-5%) and similar to those seen with interval placement at 6 wk postpartum[29,34,36,40]. Additionally, placement that occurs greater than 10 min after delivery of the placenta is associated with higher rates of expulsion than placement less than 10 min after placental delivery[31,41,42]. These findings appear consistent across multiple types of IUDs, indicating that the question of which IUD to place should be made based on patient preference and IUD availability. While the risk of expulsion may be higher with immediate postplacental IUD insertion, this risk must be weighed against the patient's risk of not returning for interval insertion. For many women with minimal access to care, the expulsion risk is worth taking. While specialized training is needed to place IUDs in the immediate postpartum setting, short didactic sessions with residents have demonstrated excellent outcomes[43].

The placement of contraceptive implants in the postpartum period is more straightforward. The implant can be inserted at any time during the hospital stay. The insertion technique and associated risks with placement in the immediate postpartum period are no different than those associated with interval placement.

PROVISION OF IMMEDIATE POSTPARTUM CONTRACEPTIVES PREVENT RAPID REPEAT PREGNANCY

Multiple studies demonstrate that contraceptive continuation rate at 6 mo and at one year post-delivery are higher among women who received LARC in the immediate postpartum period than in women who receive delayed LARC placement at the six week postpartum visit[29,35,37,44,45]. More impressive is the data demonstrating that provision of immediate postpartum contraceptive implants decreased repeat pregnancy rates at one year postpartum, despite the fact that 14% had discontinued the implant at 12 mo postpartum[10]. When evaluating women who wanted permanent contraception in the immediate postpartum period, women who did not undergo a tubal ligation had higher rates of pregnancy when compared to women who did not desire permanent sterilization postpartum despite both groups having similarly low attendance at the postpartum visit[46].

WOMEN ARE SATISFIED WHEN PROVIDED IMMEDIATE POSTPARTUM CONTRACEPTION

Women who receive immediate postpartum contraception are as satisfied or more satisfied with their methods than women undergoing delayed insertion[29,39]. When using the continued use of contraceptive method as a marker for patient satisfaction, we likewise find that more than 80% women receiving immediate postpartum LARC continue using it a year after placement[35,37,44,45]. This is significantly higher than the approximately 50% continuation rate for combination oral contraceptive pills[47]. Less is known about the continuation rates of DMPA when provided in the immediate postpartum period. Finally, contraceptive side effects occur at equal rates when comparing immediate and delayed postpartum contraception initiation[48].

IMMEDIATE POSTPARTUM CONTRACEPTION IS COST EFFECTIVE

Provision of contraception has consistently been shown to be cost effective. Recently, two separate studies have found that immediate postpartum contraception is not only cost effective, but it is cost saving. Han *et al*[49] found that every dollar spent to provide immediate postpartum etonogestrel implants to adolescents saved $6.50 within two years post-delivery. These findings factored in a high discontinuation rate (14%)

among those receiving the implant in the immediate postpartum period and still noted cost savings by providing immediate postpartum implants. Washington *et al*[50] looked at immediate postplacental IUD placement and found a cost savings of $282540 per 1000 women over 2 years. Perhaps more interestingly, these cost savings persist even if the expulsion rate of immediate postplacental IUDs is inflated to 38%.

WHY THE DELAY?

The medical evidence regarding health, safety, cost and patient satisfaction supports the use of immediate postpartum contraception. Yet, several barriers prevent the wide adoption of immediate postpartum contraception. In many locations, access to contraceptives and access to healthcare providers trained to provide contraceptives limits the ability to provide this service. Additionally, as many hospitals are owned by religiously affiliated organizations, there are more restrictions placed on what contraceptives, if any, may be provided to women. From an ethical perspective, every woman considering her delivery hospital options should be informed during her prenatal care of any deliberate institutional policies that would prohibit her access to postpartum contraception. Failure to do so is equivalent to sending a trauma victim to a hospital without emergency services. Regarding permanent contraception, women receiving state or federally funded health coverage must sign consents for the procedure at least 30 d in advance. Again, this presents a great challenge for women lacking access to routine health care as it requires not only that the patient have initiated prenatal care early in pregnancy, but also that her provider discussed contraception, including sterilization, in a timely manner. Additionally, the delivering provider must have a copy of this consent at the time of delivery in order to provide sterilization. Finally, the surgical staff must available and willing to provide the procedure. These logistical challenges reduce the chances a women will obtain a tubal ligation. Research shows only 54% of women requesting postpartum tubal ligation obtain the procedure. Of those that did not undergo tubal ligation, 37% identified problems with informed consent paperwork as the reason[51,52]. Perhaps the greatest barrier to the provision of immediate postpartum contraception is the lack of reliable hospital reimbursement. Most insurance companies in the United States reimburse for prenatal care, delivery and postpartum care as a global package. This ensures that, while tubal ligations are covered by insurance during the inpatient stay, any LARC device placed during hospitalization is not covered by insurance companies. Indeed, only placement in the outpatient setting during the postpartum visit is reimbursable.

Change within the healthcare system is difficult and slow, but it is possible. Two barriers in particular seem ripe for transformation. First, medical providers are being better educated about both the safety of immediate postpartum contraception and the actual technique of providing postplacental IUDs. Contraceptive implants require only that the provider undergo a brief 2-3 h training course for certification. As referenced above, surveys demonstrate there is still a lack of knowledge among providers and their staff regarding LARC, but there has been improvement. More recent graduates generally are more knowledgeable about immediate postpartum contraception and LARC. Clinicians will need to remember to include counseling on contraception, including sterilization, during prenatal visits so that a plan can be in place for each patient at the time of delivery. Second, the payment scheme for delivery needs to be altered to allow hospitals to be reimbursed for immediate postpartum contraception. This has been accomplished in eleven states in the United States by creating a separate Medicaid billing code for immediate postpartum contraception coverage.

CONCLUSION

The evidence clearly demonstrates the need for improving the provision of immediate postpartum contraception, given its safety, high continuation rates, low failure rates and high satisfaction. Most importantly, providers need to be vocal advocates. We need to advocate for what is right for the patient and eliminate outdated practices that are clearly inferior. We need to press for immediate postpartum contraceptive coverage by all third party payors in all states so that women can receive the best care regardless of where they live and what insurance they have.

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EDITORIAL

Avoiding misdiagnosing an early intrauterine pregnancy as an ectopic pregnancy

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Abstract

In women at risk for an ectopic pregnancy, every effort should be made to exclude the presence of an intrauterine pregnancy before embarking on an irreversible treatment for ectopic pregnancy. The diagnosis of ectopic pregnancy, unless directly visualized with transvaginal ultrasound, is made with the exclusion of an intrauterine pregnancy. Measurement of human chorionic gonadotrophin and progesterone levels, and transvaginal ultrasound are the tools used to evaluate early pregnancy. In women at risk for an ectopic pregnancy, every effort should be made to exclude the presence of an intrauterine pregnancy before embarking on an irreversible treatment course. Methotrexate is an antimetabolite that inhibits DNA synthesis and repair and cell replication. It is administered to ostensible destroy a pregnancy, especially ectopic pregnancies. When administered to an intrauterine pregnancy, embryonic death and missed abortion is the most common result, but early embryos that survive this exposure are likely to have multiple anomalies. The mistaken administration of methotrexate to an intrauterine pregnancy is made because of misinterpretation of the discriminatory zone of human chorionic gonadotropin (hCG), misinterpretation of early hCG serum levels, misinterpretation of early transvaginal ultrasound images, and failure to clinically correlate hCG levels and ultrasound findings.

Key words: Ectopic pregnancy; Ultrasound; Human chorionic gonadotropin; Methotrexate

Core tip: In women at risk for an ectopic pregnancy, every effort should be made to exclude the presence of an intrauterine pregnancy before embarking on an irreversible treatment course. Methotrexate is an antimetabolite that inhibits DNA synthesis and repair and cell replication. It is administered to ostensible destroy a pregnancy, especially ectopic pregnancies. When administered to an intrauterine pregnancy, embryonic death and missed abortion is the most common result, but early embryos that survive this exposure are likely to have multiple anomalies.

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INTRODUCTION

In women at risk for an ectopic pregnancy, every effort should be made to exclude the presence of an intrauterine pregnancy before embarking on an irreversible treatment for ectopic pregnancy, such as the administration of methotrexate. When methotrexate is administered to an intrauterine pregnancy, embryonic death with missed abortion is the most common result, but case reports of methotrexate embryopathy have described embryos that have survived early methotrexate exposure.

LITERATURE RESERACH

This is a clinical perspective from experience with cases when methotrexate has been mistakenly administered to an undiagnosed intrauterine pregnancy. Included is a review of serum hCG and progesterone levels and ultrasound interpretation.

Clinical characteristics

The diagnosis of ectopic pregnancy, unless directly visualized with transvaginal ultrasound, is made with the exclusion of an intrauterine pregnancy. The confirmation of an intrauterine pregnancy with transvaginal ultrasound relies upon recognition, initially of a true gestational sac, followed soon thereafter, by recognition of structures within the sac consistent with a developing embryo. The term "gestational sac" is a sonographic term and not an anatomical structure. A true gestational sac has a thick echogenic rim, a trophoblastic decidual reaction, surrounding a sonolucent center, the chorionic sac[1]. The intradecidual sign is the presence of such a sac buried beneath the surface of the endometrium, appearing eccentrically positioned within the endometrium (Figure 1). A "pseudosac" is a collection of fluid within the endometrial cavity itself, created by bleeding from the decidualized endometrium associated with an extrauterine pregnancy implantation (Figure 2). The precise location of such an early sonolucent uterine fluid collection should distinguish between a true gestational sac and a pseudosac.

Prior to the recognition on a definite intrauterine pregnancy, transvaginal ultrasound measurement of the endometrial echo thickness in early gestation can be helpful in predicting pregnancy location. Spandorfer *et al*[2] reporting on 117 pregnancies with a gestational age between 5.3 and 6.3 wk found statistically different endometrial echo thicknesses between patients who eventually had normal intrauterine, failed intrauterine, and ectopic gestations. Patients with normal pregnancies had endometrial echo thicknesses of 13.42 ± 0.68 mm. In contrast, those with failed intrauterine and ectopic gestations measured 9.28 ± 0.88 mm and 5.95 ± 0.35 mm, respectively ($P < 0.01$). In this report, 97% of patients with an echo no greater than 8 mm had abnormal pregnancies, and 71% of these abnormal pregnancies were ectopic in location. Only 41% of those patients with an echo thickness greater than 8 mm were abnormal, and only 14.7% were ectopic in location. No patient with an endometrial echo thickness greater than 13 mm had an ectopic pregnancy, and no patients with an echo thickness less than 6 mm had a normal pregnancy. Other authors have not been able to duplicate these discrete, well-stratified echo thickness separations[3-5]. Therefore, such absolute endometrial thickness numbers cannot be absolutely relied upon, but attention to endometrial thickness can be helpful in predicting pregnancy location, especially when the endometrial thickness is very thin or very thick.

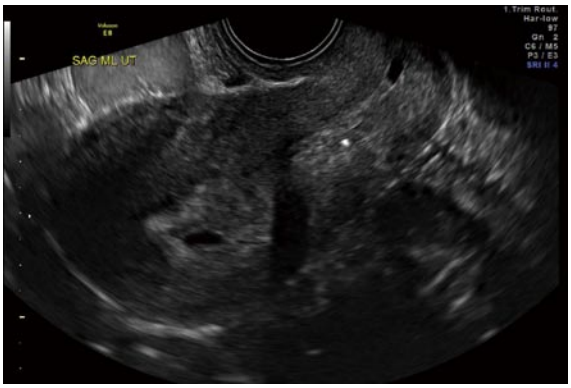

Figure 1 Transvaginal ultrasound image of a gestational sac consistent with an early intrauterine pregnancy.

The "discriminatory zone" of human chorionic gonadotrophin (hCG) is that level of serum hCG at which a normal and singleton gestation can be visualized within the endometrial cavity with transvaginal ultrasound. Depending upon the lab and the reference standard used, that hCG level is 1500 to 2000 mIU/mL. A failing pregnancy, including an ectopic implantation, should be considered, but is not confirmed, when this hCG threshold is reached and an intrauterine gestational sac is not seen with transvaginal ultrasound[6].

The possibility of ectopic pregnancy is frequently considered before hCG has reached the discriminatory zone and before ultrasound recognition[7]. Human chorionic gonadotropin rises exponentially in early normal pregnancy and should rise at least by 53% in 48 h[8]. This exponential rise is less reliable after 10000 mIU/mL, and at this level pregnancy is better evaluated with ultrasound. Fifteen percent of normal intrauterine pregnancies can demonstrate an abnormal early rise of hCG, but for the majority of gestations, when the hCG rise is abnormal, at a plateau, or falling, an abnormal pregnancy is confirmed, but not its location[9].

When exact pregnancy dating is available, an intrauterine pregnancy, regardless of embryonic number,

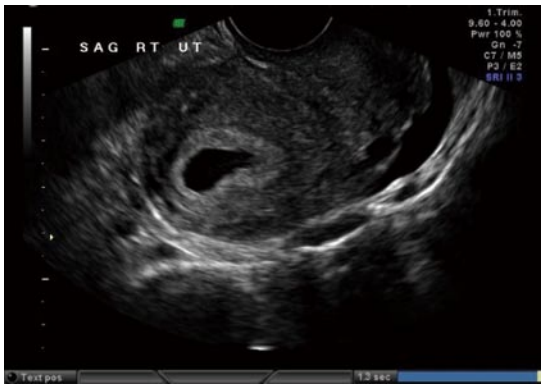

Figure 2 Transvaginal ultrasound image of a fluid collection within the endometrial cavity, a pseudosac, consistent with an extrauterine pregnancy.

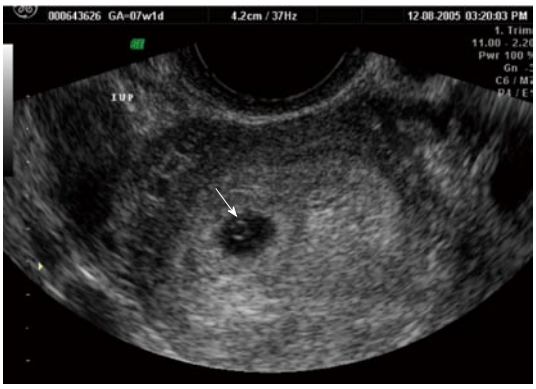

Figure 3 Transvaginal ultrasound image of an intrauterine true gestational sac containing a yolk sac.

should be identified within the endometrial cavity with transvaginal ultrasonography by 24 embryonic days, or 38 menstrual days (exact 28 d menstrual cycle)[10]. This exact pregnancy dating does not rely on human chorionic gonadotropin levels. Therefore, failure to identify an intrauterine pregnancy with such exact dating is presumptive evidence of a failing pregnancy, which could be ectopic in location. Without such exact pregnancy dating, and with no intrauterine pregnancy identified with transvaginal sonography: the "non-diagnostic ultrasound", a serum level of hCG is needed for ultrasound interpretation[10].

Usually, the transvaginal ultrasound identification of an intrauterine pregnancy reliably excludes an extrauterine implantation. A yolk sac is the first visible structure within the gestational sac, and is a distinct circular structure with a bright echogenic rim and sonolucent center (Figure 3), and is recognized 3 wk post-conception (5 wk after the last menstrual period). The embryo is first recognized as a thickening along an edge of the yolk sac, and embryonic cardiac motion can be first observed 31/2 to 4 wk post-conception (51/2-6 wk after last menstrual period).

An exception to the above is the presence of a heterotopic pregnancy: the co-existence of an extrauterine implantation with an intrauterine pregnancy. Traditionally, the incidence of heterotopic pregnancy in spontaneous cycles has been reported to be 1 in 30000 pregnancies[11]. However, its incidence is actually 1 in 3889 pregnancies[12]. The incidence is even higher (1 in 100 pregnancies) in women conceiving with *in vitro* fertilization and embryo transfer[13]. Should a clinical presentation or abnormal pelvic ultrasound appearance suggest an ectopic pregnancy, despite visualization of an intrauterine gestation, the diagnosis of heterotopic pregnancy should be considered, with the probable need for diagnostic laparoscopy confirmation and treatment.

A serum progesterone level can be helpful when the hCG is elevated, the endometrial echo is thickened and no gestational sac is seen. In a study spanning several years and yielding 3674 consecutive emergency room pregnant women visits, a serum progesterone level less than 5 ng/mL had a specificity of nearly 100% in detecting a failing pregnancy[14,15]. Therefore, with this low progesterone level, uterine curettage can be used to differentiate between a failing intrauterine pregnancy and an ectopic without the fear of interrupting a normal intrauterine pregnancy. When chorionic villi are seen on pathology, the diagnosis is a failed intrauterine pregnancy. If no chronic villi are retrieved from the uterus, the pregnancy is somewhere else. An exception to this would be with a history of heavy vaginal bleeding consistent with a completed spontaneous pregnancy loss. Therefore, after uterine evacuation and absent chorionic villi, hCG should be re-measured. A falling hCG level would support the diagnosis of a spontaneous loss, and continued observation is recommended. With a post uterine evacuation persistently elevated hCG, ectopic pregnancy is confirmed.

Methotrexate is an antimetabolite that binds to the catalytic site of dihydrofolate reductase, interrupting the synthesis of purine nucleotides and the amino acids serine and methionine, thus inhibiting DNA synthesis and repair and cell replication. It affects actively proliferating tissues such as bone marrow, buccal and intestinal mucosa, respiratory epithelium, malignant cells, and trophoblastic tissue. Systemic methotrexate has been used to treat gestational trophoblastic disease since 1956 and was first used to treat ectopic pregnancy in 1982[16]. It is administered to ostensible destroy a pregnancy, especially ectopic pregnancies. When administered to an intrauterine pregnancy, embryonic death and missed abortion is the most common result, but case reports of methotrexate embryopathy have described embryos that have survived early methotrexate exposure[17-23]. The gestational age at exposure to methotrexate is critical for the type of malformations observed[17-23]. In particular, exposure at 5 to 6 wk gestation, which is often the case in misdiagnoses of ectopic pregnancy, may lead to methotrexate embryopathy, including musculoskeletal, conotruncal, cardiac and central nervous system abnormalities[17-23]. Cardiac defects include Tetralogy of Fallot, perimembranous ventricular septal defect, patent foramen ovale, and increased pulmonary artery pressures. Intrauterine growth restriction occurs,

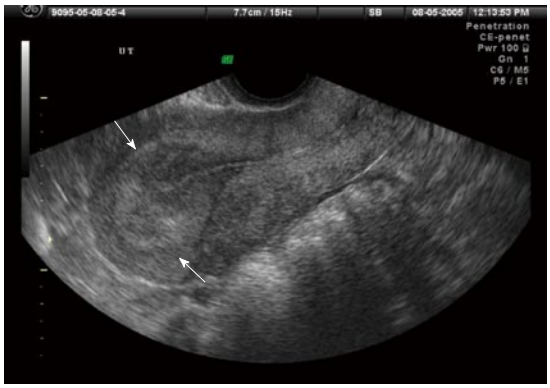

Figure 4 Transvaginal ultrasound image of a thickened endometrial echo before identification of what will be an intrauterine pregnancy.

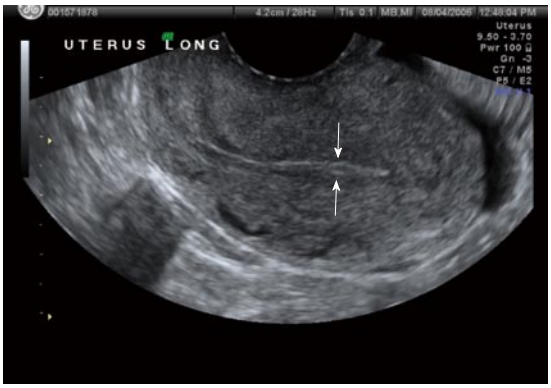

Figure 5 Transvaginal ultrasound image of a thin endometrial echo associated with and ectopic pregnancy.

and, after delivery, neurodevelopmental delay is common.

Administration of methotrexate to a woman with a presumptive diagnosis of ectopic pregnancy, which eventually turns out to have an intrauterine pregnancy, results from the following:

Misinterpretation of the discriminatory zone of hCG

As stated, the discriminatory level of hCG is defined by a "normal and singleton" gestation, *i.e.*, a single embryo pregnancy that is living and intrauterine. If a pregnancy is not seen within the endometrial cavity with transvaginal ultrasound when the hCG level has reached the discriminatory zone, an ectopic pregnancy may be suspected, but is not diagnosed with certainty. A single early hCG level is only a marker for gestational age, and clinical parameters are more important in the decision-making process. An early multiple gestation may not be seen when the singleton hCG discriminatory level has been reached, and this should especially be considered in those women who have conceived with assisted reproduction. Also, because an abnormal pregnancy would be expected to have a lower hCG level at any given gestational age, delaying the performance of a vaginal ultrasound until the hCG level has reached the discriminatory level could miss the early opportunity to diagnose an ectopic pregnancy.

Because of the variation in vaginal ultrasound technical and interpretive abilities and laboratory hCG levels, before embarking on treatment for a presumed ectopic pregnancy, especially with methotrexate, every pregnancy should be given the benefit of the doubt, even if undesired. The diagnosis may require the use of diagnostic laparoscopy for confirmation or, simply, more time and serial measurements of hCG and serial ultrasounds until the diagnosis is certain.

Misinterpretation of early hCG serum levels

Barnhart, etal, have redefined early hCG curves[8]. They report a cohort of women who presented with abdominal pain and/or vaginal bleeding, symptoms which would place these women at risk for ectopic implantation, but who eventually had normal intrauterine pregnancies. Ninety-nine percent of these early normal intrauterine pregnancies demonstrated an early hCG rise of at least 53% in 48 h. Relying on hCG measurements less than after a true 48 h interval or the use of the old concept of true doubling or a 66% rise in 48 h could misdiagnose an intrauterine pregnancy as an ectopic[10].

Misinterpretation of early transvaginal ultrasound findings

In a woman with a positive pregnancy and before the recognition of an intrauterine pregnancy with transvaginal ultrasound, endometrial echo thickness can be evaluated (Figures 4 and 5). As stated before, endometrial echo thickness can be helpful, but not definitive, in diagnosing pregnancy location: the thinner the endometrial echo, especially when less than 8 mm, the more likely the gestation is an ectopic implantation[2]. As stated before, absence of an intrauterine gestational sac on transvaginal ultrasound is not diagnostic of an extrauterine implantation, and clinical parameters must be considered in addition to absolute hCG levels, and because of the variation in the technical quality and interpretive ability of vaginal ultrasound and variation in laboratory hCG levels, the diagnoses of an ectopic pregnancy should not be made based on a single non-diagnostic ultrasound or a single hCG level. Furthermore, the location and appearance of an early intrauterine fluid collection may not always meet the criteria for a true gestational sac: eccentrically located sonolucent area surrounded by an echogenic rim.

Emergency room bedside sonography performed by emergency room personnel may differ significantly in quality and protocol from sonography performed by radiologists or trained gynecologists. A recent emergency room study reported that within a group of 161 women who were eventually proven to have a living intrauterine pregnancy, 47 (29%) were missed with initial emergency room bedside sonography with hCG levels as high as 100000 mIU/mL (mean hCG level of missed intrauterine pregnancies was 6633 mIU/mL)[21]. When the transvaginal ultrasound imaging interpretation is rendered by an emergency room physician or a radiologist, it would be prudent for the

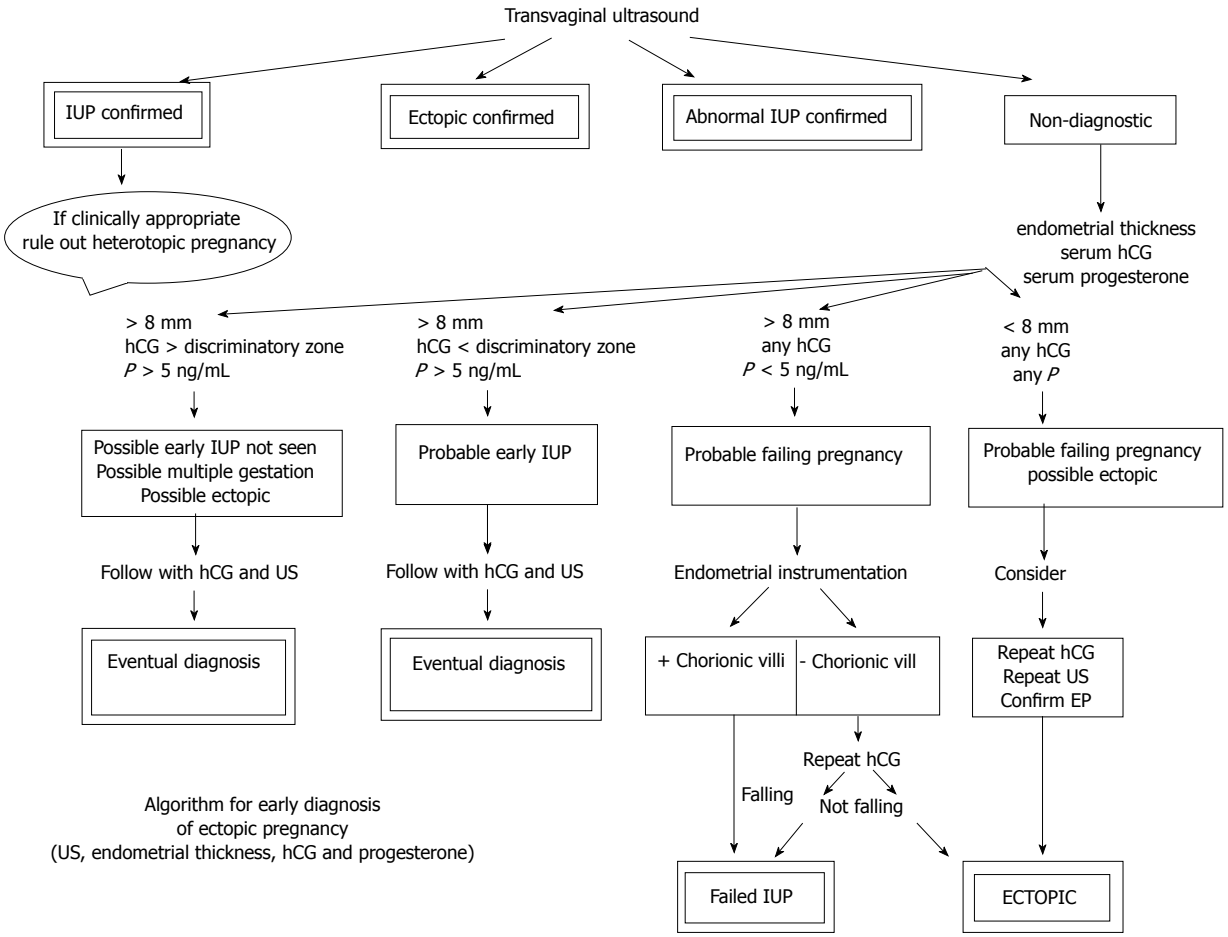

Figure 6 Non-surgical algorithm for the early diagnosis of ectopic pregnancy utilizing vaginal ultrasound, including endometrial echo thickness, serum human chorionic gonadotrophin and serum progesterone. hCG: Human chorionic gonadotropin; US: Ultrasound; EP: Ectopic pregnancy; IUP: Inverted urothelial papilloma.

treating gynecologist to view the ultrasound images and discuss the findings with the other physician before embarking of treatment of a suspected ectopic pregnancy. Unless absolutely diagnostic, caution is recommended in interpreting a single hCG level and a single ultrasound image.

CONCLUSION

The true overall incidence of methotrexate exposure to early intrauterine pregnancies is unknown and probably under reported because few cases are found in the medical literature. Every effort should be made to exclude an intrauterine pregnancy before embarking on an irreversible ectopic pregnancy treatment course, *i.e.*, methotrexate. Unless a clinical emergency exists, which would also be a contraindication to medical management, expectant management with follow up hCG levels and follow up ultrasound imaging will eventually lead to the correct diagnosis. Administration of methotrexate is never an emergency. Laparoscopy confirmation should not be disregarded when felt clinically appropriate. A thickened endometrial echo without a gestation sac needs follow up ultrasound imaging. Any intrauterine fluid collection should be considered a probable gestational sac until proven otherwise. A low serum progesterone can justify endometrial curettage to differentiate between a failing intrauterine pregnancy and an ectopic pregnancy. The rise of hCG should be interpreted in view of the new hCG curves.

Figure 6 is a suggested non-surgical diagnostic algorithm utilizing these diagnostic principles. The algorithm has not been tested to yield sensitivity, specificity, positive or negative predictive values, but adds the evaluation of endometrial thickness to the criteria of previous non-surgical algorithms. Every woman with the potential diagnosis of ectopic pregnancy should be counseled on the need for close follow up while awaiting diagnostic confirmation, rather than proceeding with an irreversible treatment with only a suspicion.

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EDITORIAL

Value of neoadjuvant chemotherapy in advanced ovarian cancer

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Abstract

Data regarding the role of neoadjuvant chemotherapy (NACT) are not definitive. Several randomized trials and meta-analyses demonstrate that this chemotherapy regimen decreases the morbidity and mortality rates and increases complete cytoreduction rates. If combined with hyperthermic intraperitoneal chemotherapy (HIPEC), NACT could potentially further improve upon these already promising results. Moreover the use of NACT could help in evaluating the chemo-sensitivity of the cancer, thus preventing unnecessary HIPEC procedures in chemo-resistant patients. NACT should definitely be considered as a preferred regimen in the management of advanced ovarian cancer, especially in association with cytoreductive surgery + HIPEC procedure in the context of a multidisciplinary team management in an experienced cancer centre.

Key words: Epithelial ovarian cancer; Neoadjuvant; Chemotherapy; Hyperthermic intraperitoneal chemotherapy; Treatment; Oncology; Cytoreductive surgery

Core tip: Data about the use of neoadjuvant chemotherapy in advanced ovarian cancer are not sufficient to support its extensive application. However encouraging results came from the existing studies. Future well designed studies are needed to clarify some aspects of this chemotherapy regimen and its association with the other form of pharmacological and surgical therapy.

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INTRODUCTION

One of the most common malignancies and one of

the principal causes of death among gynaecological neoplasm is epithelial ovarian cancer (EOC)[1]. The majority of EOC patients (about 70%) present with an advanced FIGO (International Federation of Gynecology and Obstetrics) stage disease (III or IV)[2-5]. Currently the standard treatment for these patients consists of complete cytoreduction (CC) followed by combined systemic chemotherapy of a platinum agent and paclitaxel[1,6]. Optimal cytoreduction was found to be one of the strongest survival determinants among patients with advanced stage[7-12].

NACT AND INTERVAL DEBULKING SURGERY

Recently, interval-debulking-surgery (IDS) after a short course of neoadjuvant chemotherapy (NACT), usually three cycles, has been demonstrated to be a viable alternative in those patients with low probability to obtain a CC during primary debulking surgery (PDS)[13]. Three randomized controlled trials (RCT) have demonstrated that overall survival (OS) and progression-free survival (PFS) in patients who received NACT plus IDS were not different from patients who received PDS. However, patients who received NACT had significantly lower adverse events and lower mortality after IDS than after PDS[14-16].

The first RCT, by the European Organization for the Research and Treatment of Cancer (EORTC) evaluated the benefit of IDS after suboptimal PDS. One-hundred and forty patients treated with three cycles of cisplatin and cyclophosphamide chemotherapy followed by IDS plus three cycles of ACT were compared with 138 similar patients receiving the same chemotherapy regimen without IDS. Data obtained from this study showed that patients from the IDS group had a median survival time statistically significant longer (26 mo) than patients not treated with IDS (20 mo)[14].

The second RCT conducted by the Gynecologic Oncology Group, evaluated 550 patients (stage III-IV) with a residual disease > 1 cm after PDS[15]. All patients received three cycles of initial chemotherapy with cisplatin and paclitaxel followed by response evaluation. Patients with no disease progression were randomized to IDS plus three additional cycles of ACT or additional chemotherapy alone. No differences between the two groups were found with regard to PFS or OS[15].

The third RCT performed by EORTC with the National Cancer Institute of Canada (NCIC) compared PDS with NACT plus IDS[16]. Seven hundreds and eighteen patients with EOC, fallopian tube or primary peritoneal carcinoma were included. All patients had stage IIIC-IV disease and were randomized to PDS plus platinum chemotherapy or NACT plus IDS. The CC was optimal (residual disease $\leqslant$ 1 cm) in 41.6% of patients after PDS and in 80.6% after IDS. PFS and OS were similar in both groups. Postoperative complications and postoperative mortality were higher after PDS[16].

A meta-analysis from Bristow *et al*[17] showed poor results for NACT used instead of PDS in advanced EOC. However this meta-analysis also demonstrated increased survival with an easier IDS prior to NACT and decreased survival with increasing number of chemotherapy cycles prior to IDS. Chua *et al*[18] suggested that the treatment of advanced EOC should primarily involve a massive surgical effort for CC, and NACT may be considered when the extent of the disease decreases the possibility of achieving a CC[1]. Another meta-analysis by Kang and Nam[19] showed a positive correlation between use of NACT and increased rate of CC in patients at high risk for suboptimal debulking and/or unfavourable general conditions.

Tangjitgamol *et al*[20] stated in a third meta-analysis that no conclusive evidence could be obtained to determine whether NACT increased or decreased survival rate.

NACT AND CRS PLUS HIPEC

Extensive data from the last ten years demonstrate that improved long-term results can be achieved in select patients using cytoreductive surgery (CRS), including parietal and visceral peritonectomy procedures, in combination with intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC)[18,21-30].

Data from the literature are encouraging though not entirely homogeneous[31]. Nevertheless, as stated by Markman[32], the absence of phase-III trials suggests a few considerations before definitively validating CRS plus HIPEC as a viable strategy for first-line treatment of advanced EOC[1].

While the majority of patients with EOC (up to 80%) respond to the first-line platinum based chemotherapy, almost 20% of patients are resistant or refractory[1,33]. The greatest risk is for patients requiring CRS plus HIPEC[1]. CC is associated with high postoperative morbidity and mortality rates especially in advanced cases[9,34,35]. This could potentially be increased by HIPEC as it remains a burdensome procedure. For this reason, the goal would be to select patients suitable to achieve the maximum benefit and to reduce the need for surgical resections[1]. Even if NACT followed by CRS plus HIPEC does not show better results in terms of PFS and OS[16,36], the evaluation of the NACT response may help in selecting for HIPEC-only patients who demonstrate chemo-sensitivity. In fact, NACT could have the additional benefit of providing the "*ex-iuvantibus*" chemo-sensitivity determination[1]. HIPEC with platinum compounds and taxanes in fact has been demonstrated as feasible and safe[30,37].

The addition of NACT to the current treatment regimen as documented in the literature provides some advantages with regards to morbidity reduction and completeness of cytoreduction, especially in preoperatively well-staged patients. As CC is one of the strongest predictors of survival, it is not yet well-understood why studies have failed to show an improvement in OS or DFS with NACT[7]. Nevertheless,

NACT shows great promise in its potential to prevent unnecessary use of HIPEC and to reduce surgical load, thus decreasing post-operative morbidity and mortality.

CONCLUSION

The use of NACT in the treatment of advanced EOC is progressively increasing. Studies about its use in several setting are on-going. This chemotherapy regimen should be considered as a preferred regimen in the management of advanced EOC, especially when combined with CRS plus HIPEC procedure in the context of a multidisciplinary team management in an experienced cancer centre. Results from the on-going RCT will clarify several issues about the association and the real survival effects of NACT associated to CRS plus HIPEC. Future well-designed studies are needed to clarify some aspects of this chemotherapy regimen and its association with the other form of pharmacological and surgical therapy.

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Single incision slings: Past, present, and future

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Abstract

Pubovaginal slings have become the gold standard to treat stress urinary incontinence. Traditionally, the sling referred to a suspensory that was placed under the urethra and brought through the retropubic space and anchored on either side of the midline. Since this original concept, there have been many materials used for the sling, and there have been many different anchoring approaches. Most agree that one of the best materials is polypropylene mesh. However, the means of anchoring the device and where best to have this anchorage placed is debatable. The options for anchoring simply include using darts *vs* not to hold the sling in place. The location of this anchorage, on the other hand, is much more controversial. The main locations are retropubic, transobturator, and *via* a single incision. The obturator and retropubic slings have become the standard of care over time. The single incision sling, on the other hand, is starting to be more acceptable which has resulted in it being used more frequently. The single incision relies on mainly anchoring the sling through the obturator internus muscle with possible inclusion of the obturator membrane. The purpose of this review article is to present the data that exists for the use of the single incision sling.

Key words: Sling; Stress urinary incontinence; Incontinence; Single incision sling; Surgery

Core tip: Polypropylene slings have become the mainstay of therapy for treating stress urinary incontinence in women. Historically, these slings have worked well, but there was always the concern of morbidity. The goal of the single incision sling (SIS) is to provide high efficacy with minimal side effects. The initial use of the SIS was mottled by confusion with the techniques for deployment. The most recent data has shown that when the SIS is used appropriately the success rates are similar to standard mid-urethral slings with minimal risk of bladder, vascular, or nerve injury as well as chronic pain.

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INTRODUCTION

Pubovaginal slings have been used for decades. However, it wasn't until the mid to late 1990's that the use expanded. This expansion was due in part to the use of polypropylene mesh. It was Ulmsten *et al*[1] who proved to the medical community that one could correct stress urinary incontinence (SUI) by using a piece of polypropylene mesh. Additionally, at the same time the synthetic sling became available, there was an enormous push by the device companies to educate the physicians. This education did not only include Urologists who were the main surgeon providing slings to their patients but it included gynecologists. This initially involved using transvaginal tape through the retropubic space. Although this worked well, there still was the potential for adverse events involving the bowel, bladder, and vascular structures[2,3]. Most of these complications were due to the use of trocars in the retropubic space. The transobturator sling was an evolutionary advancement, which attempted to preserve the high success rates of retropubic polypropylene slings while minimizing the chance of surgical complications. This sling in theory eliminated the chance of bowel injury and significantly reduced the chance of bladder injury. However, it still proved to possibly cause vascular injury to the obturator vessels or nerve injury to the obturator nerve. These patients were also at risk of groin pain either from muscular or tendon injury or perhaps neurologic irritation. Also, the medical community was looking for a sling that was the least invasive with high success rates and minimal chance of complications. In response to these desires, a polypropylene sling using a single vaginal incision was created.

The single incision sling (SIS) technique enables the user to place a piece of polypropylene mesh through a single vaginal incision. The idea of a SIS was first used approximately 7 years ago. The sling material varied in lengths from 8-9 cm. Some of these slings used fixation anchors while others relied more on scaring to provide fixation. Throughout the years, there were even variable length slings developed. The techniques for placement of many of the previous SISs were not consistently uniform. As a result, the early data for the SISs were not always comparable to those seen with transobturator and retropubic slings. However, the most recent retrospective and prospective studies on the use of second-generation SIS systems have demonstrated relatively high success rates with minimal morbidity. This review will provide evidence in support of the SIS.

SURGICAL TECHNIQUE

To enhance the understanding of the SIS, it is important to understand how it is placed. The description below provides the generalized technique for the placement of the SIS.

Prior to the surgery, IV antibiotics are administered. The patient is then given either local, general, or regional anesthesia at the discretion of the surgeon in combination with the anesthesiologist. A dorsal lithotomy position is then achieved to facilitate surgery. A foley is inserted to empty the bladder. A 1-2 cm anterior vaginal wall incision is made at the level of the midurethra. The dissection is than carried out laterally to the level of the inferior pubic rami on either side using blunt and sharp dissection. This surgical preparation provides a pathway for the delivery of the sling arms. The polypropylene mesh tip is placed onto an introducer, which is inserted into the dissected pathway and used to pass the distal arm anchors through the obturator internus muscle behind the pubic ramus. The sling is advanced using the introducer until the midline of the sling reaches the patient's midline under the urethra. This placement of the sling tip is repeated similarly on the opposite side. The polypropylene mesh sling is then brought to rest under the midurethra in a tensionless fashion. The anchors of the sling are resting in the obturator internus muscle. The goal of the surgeon is to visually see the periurethral tissue "pillowing" through the mesh material with a potential space existing between the sling and urethra such that a small instrument could easily be inserted. Cystoscopy is performed to ensure the bladder, urethra, and ureters are not compromised. The vaginal incision is then closed with a running absorbable suture.

CLINICAL STUDIES

There have been a tremendous number of articles written on the Single Incision technology. The early articles using SIS were mixed, and most early findings pertaining to their efficacy did not show equivalence to the results of the transobturator and retropubic slings[4]. Walsh[5] showed in 2011 that the use of the TVTsecur sling resulted in cure rates of 76% both subjectively and objectively. He described using both a "U" approach and a hammock approach. He concluded that more studies are needed before TVT secure could be routinely used. There were other slings such as the Ajust sling by C.R. Bard, Inc., New Providence, NJ United States, that conceptually made sense and, if used in the appropriate hands, yielded high success rates. In Jiang *et al*[6] paper, he showed that using the AJust sling resulted in subjective and objective sure rates of 82.3% and 91.2% in a 12-mo follow-up respectively. This was a single site study where there were no cases of bladder perforation or major bleeding. There were also no reported cases of groin pain at 6 and 12 mo[6].

This study exemplifies the importance of technique when placing the SISs. Although this group of researchers was able to achieve high success rates with this sling, the sling was not universally deployed successfully, and, as a result, this sling even with its high success rates is no longer being marketed by C.R. Bard Inc.

Initially, the SIS was thought to work differently than other slings and its placement and tensioning were not standardized. Surgeons were using it to go in the

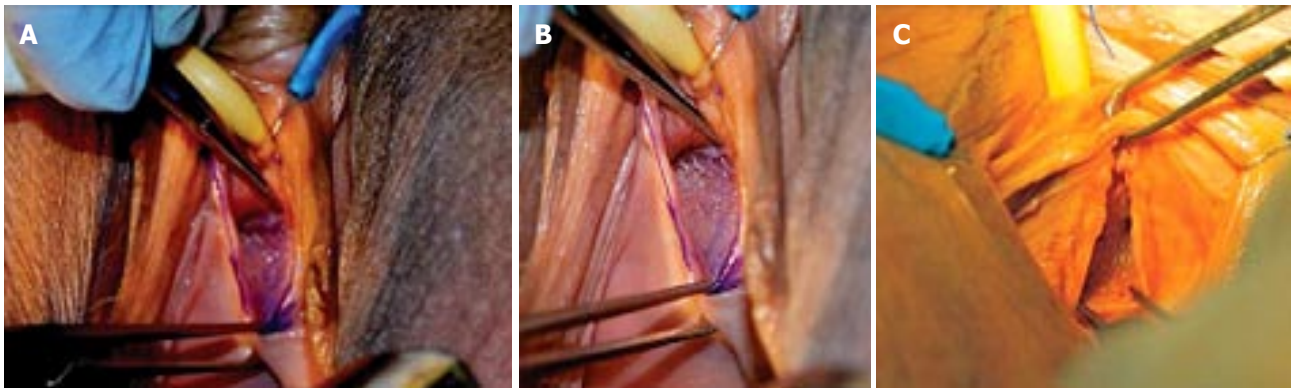

Figure 1 Placement of mid-urethal slings to alleviate stress urinary incontinence using the (A) retropubic; (B) transobturator; (C) single incision techniques.

retropubic direction as well as the obturator location. It then became accepted by most that the placement was to be in the obturator internus muscle. The tension could be set in many different ways, but the end result would be a sling that was up against the urethra with the periurethral tissues "puckering or pillowing" through the mesh openings such that a potential space existed to insinuate a small medical instrument between the urethra and the sling (Figure 1).

In the article entitled "Cadaveric Assessment of Synthetic Mid-Urethral Sling Placement", the placement of the SIS was compared to the obturator and retropubic sling[7]. It was determined that the SIS was similar to the others in appearance and furthermore was most likely at the midurethra and had the most correct tension. It is studies like this that show what is being done by the three sling approaches have different means of achieving the same endpoint.

There are presently around 26 randomized controlled trials, which are using 7 different types of SISs. In these studies approximately 3300 patients were evaluated[8].

Many of the studies have been performed comparing the SIS to the standard mid-urethral slings, which are considered to be either obturator slings or retropubic slings. The majority of these studies support the use of the SIS[9-25]. Lee *et al*[25] recently published a randomized trial comparing single incision *vs* outside-in transobturator mid-urethral sling. This paper studied the MiniArc SIS and showed an objective cure rate of 94.4% and a patient reported cure of 92.2% at 12 mo. The Monarc sling was the comparator to the MiniArc and it showed statistically similar results with a 96.7% objective sure and a 94.2% subjective cure. The operative time was reduced by 0.5 min in the SIS group. The Monarc group required more analgesia in the first 24 h and reported more short-term groin pain. The quality of life questionnaires and sexual function questionnaires revealed similar results in both groups. The patients undergoing repeat incontinence surgery were 2.7% in the MiniArc group compared to 1.8% in the Monarc group while 6.2% of the Monarc group had groin pain beyond 6 mo compared to 0% in the MiniArc group. For both patient groups, BMI and age were associated with higher failure rates[25].

Similar data was shown by Enzelsberger *et al*[26] who also looked at the MiniArc SIS and compared it to the Monarc. In this study, there was an objective cure rate of 82%. They also had shorter OR times and less groin pain. In our long-term study using the Solyx SIS, we also saw subjective success rates of 93% over a mean follow up of 43 mo[27]. There was, however, one recent article by Basu *et al*[28] that showed a lower success rate with the SIS than an obturator sling. This study also had a higher erosion rate with the SIS, which possibly implies a technical issue with using the single incision technology[28].

In the Mostafa *et al*[8] metaanalysis, he primarily looked at the MiniArc sling as compared to either retropubic or obturator slings. This study shows an aggregate objective cure rate of 88% with a subjective cure rate of 76% for the SISs. Additionally, the SIS had shorter operative times, lower incidence of groin pain, earlier return to work, and lower pain scores. There were no significant differences in subjective or objective cure rates for the SIS *vs* the standard mid-urethral slings. Also, the impact on quality of life and sexual function were similar. The TVT secur was not included in this analysis due to its poor early data and that it is now off the market as of 2012.

CONCLUSION

There are many SISs currently available. Each is different in its design and applicator as well as technique for placement. They all hope to provide the same endpoint, which is a backboard for the urethra to use with increases in abdominal pressure. Current data does suggest that if the SIS is used appropriately there would be an enhanced safety profile with less postoperative discomfort and high success rates. It is the responsibility of the medical community to provide guidelines for the use of these slings and to standardize their placement to assure reproducibility of the success rates. The correct use of SISs will ultimately lead to a treatment, which provides high success rates with low morbidity for our patients who suffer with SUI.

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EDITORIAL

Infertility and ovarian failure in celiac disease

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Abstract

Unexplained infertility in females may be a devastating event for the reproductive-aged female. However, infertility may be due to ovarian failure associated with celiac disease, an immune-mediated disorder that may have few or no symptoms and can be successfully treated. In some prospective serologically-based studies, over 4% of infertile females may prove to have celiac disease. Serological screening for celiac disease is relatively inexpensive and involves testing for antibodies to tissue transglutaminase. If positive, a small intestinal biopsy should be done to confirm the diagnosis. The initial treatment for this disorder is a gluten-free diet. To date, a number of reports have indicated that this treatment for celiac disease may result in successful pregnancy, in spite of prolonged periods of infertility. Celiac disease, when untreated, may also lead to several adverse events following pregnancy including increased risk of recurrent abortions, low birthweight and impaired fetal growth. Recent molecular and pathological studies from different laboratories suggest that altered placental function may be due to binding to cells in the trophoblast by tissue transglutaminase antibodies impairing embryo implantation and leading to failure of early pregnancy or retarded intrauterine growth.

Key words: Celiac disease; Infertility; Ovarian failure; Autoimmune disease; Polyglandular syndrome

Core tip: Females with unexplained infertility should be screened for celiac disease. This involves use of a simple and inexpensive serological quantitative method for detection of tissue transglutaminase antibodies, a marker for celiac disease. If positive, biopsy evaluation should be done to determine if pathological features of untreated celiac disease are present in the small intestinal mucosa. A gluten-free diet may lead to effective management of celiac disease and may promote a favorable pregnancy outcome.

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INTRODUCTION

Infertility is a significant, even devastating clinical issue for some reproductive-aged women. Although infertility

may result from a number of factors, premature ovarian failure may be responsible. Ovarian failure normally occurs with the process of aging, premature failure of ovarian function may be defined as the failure of estrogen production by the human ovaries, usually before the age of 35 to 40 years. There may also be other significant long-term health consequences associated with premature ovarian failure including osteoporosis, heart disease, autoimmune disorders and increased risk of mortality[1]. There are many causes of premature ovarian failure in adults that need to be considered, including celiac disease.

FACTORS ASSOCIATED WITH FEMALE INFERTILITY

A number of different factors may lead to investigation of altered female fertility and, traditionally, these may be considered anatomically to include local factors in the cervix (*e.g.*, altered cervical mucus) and uterus (*e.g.*, congenital uterine abnormalities), fallopian tubes (*e.g.*, adhesions) as well as ovarian failure. Ovarian failure *per se* may have several causes, including polycystic ovary syndrome, hyperprolactinemia or hypothalamic amenorrhea, often associated with pituitary disorders, including tumors, or some medications. Ovulatory dysfunction may also occur in association with endocrine disorders, particularly those associated with an altered autoimmune process, including hypothyroidism or adrenal insufficiency (if together, Schmidt's syndrome, and when coupled with diabetes, Carpenter's syndrome). Either of these, but particularly autoimmune thyroiditis, may be frequently observed with celiac disease. Alternatively, an autoimmune polyglandular syndrome affecting several endocrine tissues can lead to ovarian failure. Polyendocrinopathy-candiasis-ectodermal dystrophy syndrome (APECED) is considered a rare autosomal recessive disease caused by mutations in the autoimmune regulator (*AIRE*) gene. Typical clinical findings include candidiasis, Addison's disease, hypoparathyroidism, diabetes, alopecia, vitiligo, ectodermal dystrophy, autoimmune thyroiditis, pernicious anemia, chronic active hepatitis, celiac disease and premature ovarian failure[2].

CELIAC DISEASE

Celiac disease is an immunologically-mediated gluten-sensitive small intestinal mucosal disorder primarily detected in genetically-susceptible persons[3,4]. Gluten peptides found in grains appear to be a triggering environmental factor following dietary exposure. Mechanisms involved in the pathogenesis of celiac disease are beyond the scope of this article, but have been recently detailed[4]. Chronic diarrhea, malabsorption of major and minor nutrients, and weight loss may occur. Development of serum antibodies to tissue transglutaminase also occurs in celiac disease and quantitation of these antibodies is a useful method for serological screening of populations or case finding in clinical practice. Endoscopic biopsies of the proximal small intestinal mucosa in untreated celiac disease show typical inflammatory changes along with moderate to severe alterations in mucosal architecture. Usually, these clinical, serological and pathological changes in untreated celiac disease respond to a gluten-free diet. Recognition of celiac disease is important because of the potential for later development of other co-morbidities, including osteoporosis and malignancy. Extra-intestinal or autoimmune processes may also occur, including dermatological changes, such as dermatitis herpetiformis, and endocrine disorders, including hypothyroidism associated with autoimmune thyroiditis. Often, these extra-intestinal features may be the presenting symptoms to an underlying small intestinal mucosal disorder that may be clinically silent.

CLINICALLY SILENT CELIAC DISEASE

Celiac disease has now become more often appreciated to have few or limited intestinal symptoms. Minimal diarrhea without weight loss may be evident. Often, females with celiac disease and reproductive disorders have no overt symptoms, or perhaps, only decreased energy with iron deficiency or, if more significant, iron deficiency-associated anemia[5]. As a result, impaired female fertility or changes that include delayed onset of menses, amenorrhea and early menopause may conceivably be the initial clinical presentation that eventually leads to recognition of underlying celiac disease. Owing to the increased ability to serologically screen for celiac disease, up to 1% to 2% of individuals in the general population in some countries have been detected with this disorder, especially in women of child-bearing age[4]. Screening biopsies may also be done and these studies have demonstrated that young adult females, in particular, are the most common adult age group first diagnosed with celiac disease[6]. Conceivably, premature ovarian failure leading to infertility could be reflect underlying immune-mediated mechanisms occurring in celiac disease, or alternatively, negative nutritional consequences of impaired absorption in celiac disease *per se*.

CELIAC DISEASE AND POLYCYSTIC OVARY SYNDROME

An important consideration in the broad differential diagnosis of premature ovarian failure is the polycystic ovary syndrome, initially described by Stein and Leventhal in 1935. Since then, this syndrome has been extensively investigated, and appears to be very heterogeneous with clinical features that include menstrual changes, chronic anovulation and excessive androgen levels, often with hirsutism. Anatomically, large cystic ovaries are often detected, but in up to

Table 1 Celiac screening studies in females with infertility

Number[1]	Country	Celiac disease[2]	Ref.
150 (98)	Finland	2.7% (4.1%)	[12]
99	Italy	3.03%	[14]
192	Israel	2.65%	[15]
47	Finland	2.1%	[16]
200[3]	Italy	2.5%	[18]
51	United States	5.9%	[20]
29	Brazil	10%	[21]

[1]Number with infertility (number in parentheses, unexplained infertility subgroup); [2]Celiac disease suspected with serological screening, usually with tissue transglutaminase or endomysial antibodies, and confirmation with small intestinal biopsy; [3]Infertility group for assisted reproduction technology.

a third of women with the same clinical features, the ovaries appear to be normal. Gonadotropin secretion appears to be abnormal with elevated levels of luteinizing hormone, or LH, combined with normal or low levels of follicle stimulating hormone, or FSH. In some, a mild elevation of serum testosterone is evident. The cause of the polycystic ovary syndrome still requires clarification. An earlier study suggested that the polycystic ovary syndrome may also occur in celiac disease[7]. Unfortunately, the data needed to fully examine this relationship (even with development of easy-to-perform serological screening measures) are currently not available. Some patients labeled with the polycystic ovary syndrome, especially with normal-appearing ovaries, could also conceivably have occult celiac disease, potentially amenable to a gluten-free diet.

ALTERED FEMALE FERTILITY IN CELIAC DISEASE

First cases noted a possible relationship between celiac disease and female infertility[8,9]. In one, subsequent treatment with a gluten-free diet resulted in pregnancy, confirming observations in 2 other reports[10,11]. Later, more extensive population-based studies were done to explore this potential relationship (Table 1). A detailed serological evaluation of 150 women with infertility due to all causes from Tampere, Finland demonstrated an apparent overall rate of celiac disease (*i.e.*, 2.7%)[12], (although subsequent serological screening studies from Finland in otherwise healthy subjects have revealed a relatively high rate of celiac disease in Finland compared to other countries[13]). For women with unexplained fertility, however, this study also reported rates of detection of celiac disease of over 4%[14]. Similar results were later reported in 99 couples from Northern Sardinia[15], estimated to be a rate of approximately 3%. Using more modern serological assays, a study from Israel employed assays for both tissue transglutaminase and endomysial antibodies in 192 Arab females with unexplained infertility. Among these, positive serological tests were noted in 2.65%[15]. In all, small intestinal biopsies were positive for changes of celiac disease, if serological studies were positive. Like most serological screening studies of populations, however, biopsies in serologically negative patients were not defined. Other studies have provided different results. Interestingly, a different center in Finland[16] could not confirm earlier study results from the same country[12]. A Czech investigation reported increased serum antibody positivity for celiac disease in women with infertility, but biopsies were not reported[17]. In a study from Italy that evaluated a group of infertile women specifically referred for assisted reproduction, a significant association with serology could not be defined[18] while a Swedish population-based cohort study of biopsy-defined celiac disease suggested that fertility was not decreased until the final 2 years preceding diagnosis[19]. Data from the United States demonstrated a prevalence of celiac disease in 5.9% of patients with unexplained infertility[20], while a Brazilian study evaluated 170 infertile women screened for tissue transglutaminase antibodies followed by small bowel biopsies in serologically-positive patients[21]. In this aforementioned Brazilian study, the prevalence of celiac disease was 10.3% in women with unexplained fertility. This contrasted with a prospective primary care study of over 2 million women from the United Kingdom where no overall impairment in fertility was recorded in celiac disease compared to non-celiac disease women[22]. However, in the same study[22], infertility rates were over 40% higher in celiac disease patients between ages 25 to 29 years compared to a similar age-matched population without celiac disease. Finally, a recent report[23] describing a meta-analysis in 105 relevant studies reported that "all-cause" infertility was 3.5 times higher in women with celiac disease compared to controls while "unexplained infertility" in women with celiac disease was 6 times higher than controls. Thus, recent population-based studies of women with unexplained infertility suggest a significant occurrence of occult celiac disease. Moreover, some of these celiac patients were reported to have a successful pregnancy following treatment with a gluten-free diet.

OTHER FERTILITY-RELATED CHANGES IN CELIAC DISEASE

Delayed onset of menses, amenorrhea, early menopause, repeated abortions and diminished pregnancy rates in celiac disease could indicate a possible impairment in fertility. In 74 patients from the United Kingdom[10], the reproductive period appeared to be more prolonged for celiacs on a gluten-free diet compared to celiacs not on a gluten-free diet. Otherwise, maternal health did not appear to be significantly altered. Nonetheless, a lower incidence of spontaneous abortions was noted in celiacs treated with a gluten-free diet. These findings were supported by a subsequent study from Italy[24]. A delay in the onset of menarche

(13.5 years *vs* 12.1 years) was also noted compared to age- and "sexual behavior-matched" controls. Amenorrhea and repeated abortions were more frequent in the celiac group, but menopause onset was not affected. A Polish study[25] suggested that the age of menarche in celiacs could be regulated by a gluten-free diet, while an Italian study[26] suggested that menarche was mainly impacted by the female's maternal history of menses onset. A United Kingdom study showed that celiacs may have limited fertility with a higher incidence of stillbirths and perinatal death[27]. However, the rate of miscarriage appeared to be improved after diagnosis of celiac disease and treatment with a gluten-free diet[27]. Finally, in a report from Brazil[28], gluten-free diet therapy appeared to be instrumental in promoting a positive nutritional state, relevant to reproductive health in patients with celiac disease.

Some earlier studies have evaluated pregnancy outcomes in celiac disease[29,30]. If untreated, celiac disease is associated with an increased risk of recurrent miscarriage and premature deliveries, along with reduced fetal growth and term birthweight[29]. In an Italian study[30] of 94 untreated and 31 treated celiac patients, relative risks of either abortion or delivery of a low birthweight infant were increased while breast feeding duration was reduced. The investigators noted improvement with a gluten-free diet. Similar observations have been recorded by others[31]. Lower birthweight and retarded intrauterine growth have been recorded from European centers[32-35]. For example, in a further Italian study[33], celiac disease was more common than most diseases normally screened for during pregnancy in their health care facility. In another European evaluation[34], undiagnosed maternal celiac disease appeared to be a far greater risk factor than diagnosed celiac disease. However, a later report was not able to confirm an unfavorable pregnancy result[36]. Later studies also suggested that celiac disease in the father was not a risk factor for an adverse outcome of the pregnancy[37,38].

PLACENTAL STUDIES

The precise mechanism for adverse pregnancy outcomes are not clear but have been evaluated to a limited extent. Placentas of some mothers affected with celiac disease, in particular, may be abnormal. Using immunohistochemical methods and *in situ* hybridization, tissue transglutaminase expression and apoptosis were increased in trophoblast cells[39]. This suggested a possible injury mechanism in both fetal and placental portions of the placenta[39]. Maternal celiac disease autoantibodies may bind directly to the syncytiotrophoblast and inhibit placental tissue transglutaminase activity leading to an impaired placenta[40]. Other studies in female celiacs have raised the issue of early pregnancy loss due to altered coagulation affecting placental or fetal microvascular function[41]. Finally, binding of antibodies to tissue transglutaminase by the trophoblast might represent a crucial mechanism causing impaired embryo implantation and pregnancy outcome in pregnant celiacs[42]. Additional studies are needed to explore these intriguing early findings in celiac disease.

CONCLUSION

A preponderance of evidence from multiple studies indicates that infertility or ovarian failure in females may be increased in immune-mediated disorders, including celiac disease. In celiac disease, an immune-mediated small intestinal mucosal disorder is triggered by gluten-containing peptides found in food grains. The primary treatment of celiac disease involves administration of a strict gluten-free diet. In females with untreated celiac disease and infertility, successful pregnancy may occur solely after treatment of the celiac disease with a gluten-free diet. Widespread use of serological screening in different populations suggests that celiac disease may occur in 4% to 10% of females with unexplained infertility. Serological screening using tissue transglutaminase antibodies represents a very inexpensive method for evaluation, and if positive, should be followed by small intestinal biopsies to define the presence of celiac disease and lead to treatment.

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Preeclampsia - What is to blame? The placenta, maternal cardiovascular system or both?

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Abstract

Preeclampsia (PE) is a pregnancy-specific syndrome, complicating 2%-8% of pregnancies. PE is a major cause of maternal mortality throughout the world with 60000 maternal deaths attributed to hypertensive disorders of pregnancy. PE also results in fetal morbidity due to prematurity and fetal growth restriction. The precise aetiology of PE remains an enigma with multiple theories including a combination of environmental, immunological and genetic factors. The conventional and leading hypotheses for the initial insult in PE is inadequate trophoblast invasion which is thought to result in incomplete remodelling of uterine spiral arteries leading to placental ischaemia, hypoxia and thus oxidative stress. The significant heterogeneity observed in pre-eclampsia cannot be solely explained by the placental model alone. Herein we critically evaluate the clinical (risk factors, placental blood flow and biomarkers) and pathological (genetic, molecular, histological) correlates for PE. Furthermore, we discuss the role played by the (dysfunctional) maternal cardiovascular system in the aetiology of PE. We review the evidence that demonstrates a role for both the placenta and the cardiovascular system in early- and late-onset PE and highlight some of the key differences between these two distinct disease entities.

Key words: Preeclampsia; Placenta; Maternal cardiac function; Cardiovascular; Aetiology

Core tip: The conventional paradigm is that preeclampsia (PE) is solely due to placental dysfunction. However not all cases of placental dysfunction result in the syndrome of PE. Equally, placental dysfunction - as evidenced by impaired uterine artery Doppler indices, low birth weight and abnormal histology - is not always present in PE. Therefore, the heterogeneity observed in PE cannot be solely explained by placental dysfunction. There is now strong evidence supporting the role of the maternal cardiovascular system in PE. In this mini-review, we evaluate the evidence supporting a dual

aetiology of PE, involving both the placenta and the maternal cardiovascular system.

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Preeclampsia (PE) is a pregnancy-specific syndrome, complicating 2%-8% of pregnancies[1]. PE is a major cause of maternal mortality throughout the world. Over half a million women die during or after childbirth every year, and it is estimated that 60000 of these maternal deaths worldwide are attributable to hypertensive disorders of pregnancy[1,2]. In the United Kingdom, PE/eclampsia was the fourth leading cause of direct maternal deaths between 2009-2011[3]. In the triennium 2009-2011, 10 deaths in the United Kingdom were attributable to PE, giving a mortality rate of 0.42 per 100000[3], considerably lower than the mortality figures worldwide. The management of hypertension in conjunction with the use of national and local guidelines are credited with the reduction in morbidity and mortality observed in the developed world.

PE can also result in morbidity to the fetus, including fetal growth restriction, placental abruption and stillbirth. In addition, there is a considerable financial burden as a result of increased antenatal surveillance, investigations and hospital admissions along with an increased risk of operative intervention and peripartum monitoring. Following delivery, prematurity and the associated care costs, both in the short and long term, further contribute to the financial burdens associated with PE. At present, the only recognised cure for PE is delivery of the placenta. As a result, PE is the leading cause for iatrogenic preterm birth[4], which is associated with greater neonatal morbidity than delivery at term.

The importance and magnitude of PE on maternal health and neonatal morbidity is well accepted. Despite this being such an important disease, our knowledge of its aetiology is still incomplete. Forming a better understanding will aid us in making an accurate diagnosis, perform better screening and improve our ability to triage disease severity, offer targeted preventative and therapeutic measures and formulate appropriate short- and long-term postpartum management plans.

PE is a heterogenous disease exhibiting a diverse range of both fetal and maternal disease presentations. At present, we aim to identify and diagnose PE based on organ dysfunction both in the mother and in the fetus. The timing of onset of PE is as diverse as its organ involvement and can occur from the late second trimester, through to term and can even present in the postnatal period.

Although often classified as mild, moderate and severe, a newer and perhaps more relevant method of clinical classification would be based upon timing of onset. Early onset PE is thus defined as PE occurring prior to and requiring delivery before 34 wk gestation and late onset PE developing and requiring delivery after this gestational age. There are distinct differences between these two types of PE, in terms of their pathophysiological basis, effects on mother and fetus as well as their long term implications. Late PE accounts for approximately 75% of cases, whilst the remaining 25% are early onset PE[5,6].

Ten percent to fifteen percent of pregnant women will develop some form of hypertensive disease that requires further assessment and follow up. Currently, United Kingdom guidelines on screening for identification of those at high risk of developing PE is performed in the first trimester and is based on maternal demographics and obstetric/medical risk factors[7]. The NICE model recommends commencing preventative measures (*e.g.*, low dose aspirin) and formulation of an appropriate antenatal management plan in high risk women. However the NICE model categorises more than 60% of women as high risk, but predicts less than 30% of those destined to develop PE[8].

PATHOPHYSIOLOGY - THE ROLE OF THE PLACENTA

The precise pathophysiological basis of PE remains an enigma with multiple, plausible aetiological theories involving a combination of environmental, immunological and genetic factors.

The conventional, leading hypotheses, which has stood the test of time, for the initial pathophysiological insult in PE is inadequate trophoblast invasion and thus incomplete remodelling of uterine spiral arteries This leads to placental ischaemia, hypoxia and thus oxidative stress[9,10]. The placental hypoxaemia sets off a biochemical cascade of angiogenic/antiangionic factors which leads to subsequent endothelial cell dysfunction with its observed maternal signs and symptoms of PE. Whilst placental hypoxaemia and the subsequent oxidative stress is a significant contributing factor, we acknowledge that a definitive link between PE and placental hypoxia is not confirmed and further research is required.

However not every pregnancy characterised by abnormal placentation results in PE - fetal growth restriction is one such example in where there is suboptimal placentation with detrimental fetal effects but minimal maternal effects. Others will go on to develop the syndrome of PE which is characterised by hypertension and proteinuria and multiple organ involvement.

One possible reason for the development of PE is that there is a concurrent maladaption of the maternal cardiovascular system along with abnormal placentation, and this predisposes an individual to developing PE. This would also explain why not all women with poor placentation with its sequalae (such as fetal growth restriction) develop PE; their cardiovascular system adapts appropriately to the ongoing pregnancy and associated haemodynamic changes.

IS PE SOLELY DUE TO PLACENTAL DYSFUNCTION?

The development of PE does not necessarily require a uterus or indeed a fetus, as the condition has been reported in abdominal[11] and molar pregnancies[12] respectively. However, a placenta is essential for the disease to occur, and is therefore central in the pathogenesis. Furthermore, it is well documented that the cure for PE is delivery of the placenta, further supporting the crucial role played by the placenta in the development of PE.

The paradigm is that the "placenta causes PE". Whilst we firmly believe that the placenta is a pre-requisite and therefore crucial to the development of PE, herein we critically review the evidence that the placenta is indeed the only organ of causality in PE.

CLINICAL EVIDENCE FOR THE AETIOLOGY OF PE

The majority of the evidence for the placental origin hypothesis of PE is based on the clinical features of the disorder. Broadly speaking, they constitute clinical risk factors, placental blood flow evaluation, fetal growth restriction and placental biomarkers.

Risk factors for PE

Both PE and fetal growth restriction (FGR) are thought to share clinical risk factors such as increased maternal age, ethnic origin, increased body mass index (BMI), diabetes and other co-morbidities. These associations are conventionally advocated by national guidelines[7,13] as a method to screen routine populations for their risk of developing PE. However, analysis of data from 40000 pregnancies collected for the World Health Organization Antenatal care trial[14] showed that surprisingly, PE and fetal growth restriction had different risk factor profiles. Mothers with PE compared with those with fetal growth restriction were more likely to have a history of diabetes, renal or cardiac disease, chronic hypertension, previous PE, increased BMI and extremes of maternal age. Conversely, fetal growth restriction was associated with higher risk of low birth weight in previous pregnancies, but not with previous PE. The same analysis demonstrated that PE and gestational hypertension shared many risk factors - conventionally associated with cardiovascular disease in the non-pregnant population. These data infer that the aetiology of PE is not necessarily as a result of placental dysfunction.

Placental blood flow

The pathological hallmark of placental insufficiency is incomplete spiral artery remodelling, and this is seen in both fetal growth restriction as well as in some, but not all cases of PE. Uterine artery Doppler waveform studies have been shown to aid in the prediction of pregnancies that will be complicated by fetal growth restriction or PE[8,15-17]. Increased uterine artery resistance indices are related to incomplete trophoblast invasion of maternal spiral arteries, which results in a high-resistance placental circulation and therefore an underperfused fetoplacental unit. This placental hypoperfusion is a feature seen in both PE and fetal growth restriction.

The association with increased uterine artery Doppler resistance indices and a higher propensity to develop PE and FGR is widely reported and used in current clinical practice in the management of high risk pregnancies. Velauthar *et al*[17] carried out a meta-analysis of over 55000 patients looking at the predictive value of performing uterine artery Doppler measurements in the first trimester. The authors reported a sensitivity and specificity of 47.8% and 92.1% respectively for the detection of early onset PE. The sensitivities and specificities for detection of late onset PE were 21.5% and 90.3%; for PE at any gestation these were 26.4% and 93.4%. Although first trimester uterine artery Doppler screening is a highly specific means of screening for early PE, it is less so for late PE.

Verlohren *et al*[18] have corroborated this finding in their published data of second trimester uterine artery Doppler screening. The investigators looked at outcomes in over 27000 cases that had uterine artery measurements in the second trimester. They reported that the prevalence of resistance indices above the 90th centile were present in 63.6% of early PE, 15.5% of late PE and 8.8% in the control group. This significant finding shows that increased uterine artery Doppler indices, are a poor predictor of late onset PE.

It is well recognised that increased uterine artery dopplers are an index for inadequate placentation, and thereby a surrogate marker for increased risk of PE and FGR. Data from these large-scale, high quality studies indicate that inadequate placentation is a key feature of early onset PE and FGR. The reduced prevalence of increased uterine artery indices observed in late-onset PE lends further support to the argument that the heterogeneity observed in PE, is due to early onset PE being related to a dysfunctional placenta, whilst late onset PE may not be associated with placental insufficiency.

Fetal growth

The fetal effects of a dysfunctional placenta include fetal growth restriction, and this is seen in many cases of early-onset PE, but less so in cases of late PE. The majority of neonates in late PE are of normal size[18]. This observation is consistent with the notion that whilst early PE due to placental insufficiency is also closely correlated to FGR, there is an additional aetiological factor to placental insufficiency in late onset PE.

Verlohren *et al*[18] reported on the distribution of small-for-gestational-age (SGA) neonates in their large cohort. They found the incidence of SGA to be 66.2% in the early PE group, 16.7% in the late PE group and 10.8% in the control group.

The distribution of large-for-gestational age neonates did not observe a similar pattern; the prevalence being greatest in the late PE group as compared to all other groups. Interestingly, both SGA and large-for-gestational-age (LGA) births were more prevalent in the late PE group, demonstrating a bimodal skewed birth weight distribution in late PE. Whilst the association between PE and LGA babies has been observed previously, the bimodal distribution is certainly a novel finding.

The increased incidence of SGA births in early-onset PE, associated with increased uterine artery impedances, is an expected finding given the underlying placental insufficiency, unlike the reported increased prevalence of both SGA and LGA in late-onset PE. The authors postulate that this finding implies that whilst the SGA form of late-onset PE is due to placental insufficiency, the LGA form (with its associated normal uterine artery impedance measurements) is secondary to the inability of the maternal heart to meet the demands of the (oversized) placenta. The subsequent placental hypoperfusion as a result of the inadequate pump action, leads to placental hypoxia and the biochemical cascade that leads to endothelial cell dysfunction observed in PE.

Placental biomarkers

The oxidative stress in the placenta leads to an imbalance in angiogenic and antiangiogenic factors. An imbalance in these factors can adversely affect vascular homeostasis, and therefore contribute to the array of symptoms displayed in PE. The angiogenic factors that are believed to play a role include vascular endothelial growth factor (VEGF) and placental growth factor (PlGF)[10,19,20], the latter being used as a potential diagnostic biomarker for PE[19,20]. PlGF is produced by trophoblasts and interacts with cell surface receptors such as Flt-1. The soluble form of this, soluble fms-like tyrosine kinase-1 (sFlt-1) is an antiangiogenic factor which has also emerged as a key factor and potential biomarker in PE. sFlt-1 has been shown to block PlGF and therefore play a causative role in the development of PE[10,21]. Maynard *et al*[10] demonstrated a rise in arterial pressures and proteinuria with exogenous administration of sFlt-1. Furthermore, uteroplacental ischaemia has been shown to increase sFlt-1 levels in experimental models, as well as a decrease in VEGF/PlGF[22,23].

Another biological factor that has been studied and may have a role in PE is Heme Oxygenase-1 (HO-1). HO-1 and its metabolites carbon monoxide (CO) and bilirubin exert protective effects against oxidative stimuli, and *in vitro* studies have reported that HO-1 downregulates sFlt-1[24]. HO-1 has been shown to inhibit sFlt-1 release[24,25] and it is possible that a loss of HO-1 plays a part in the pathophysiology of PE. There are no published studies looking at the differences in the expression of HO-1 in early-onset *vs* late-onset PE.

Poon *et al*[26] conducted a first trimester screening study using uterine artery Doppler indices, serum pregnancy associated plasma protein A (PAPP-A) and placental growth factor measurements in a large cohort of over 57000 cases. They found that in the pre-term SGA group, serum PAPP-A and PlGF were reduced, indicating an underlying pathology of impaired placentation. The authors reported that for a false-positive rate of 5%, the detection of early-onset PE (< 34 wk gestation) using PlGF was 59.3%, however in all cases of PE below 42 wk gestation, the detection rate was significantly lower at 29.1% - this figure included all of the early-onset along with late-onset cases of PE. The evidence suggests that placental biomarkers have a good performance for screening for early-onset PE, but a poor performance when screening for late-onset PE.

The biomarkers discussed above have been used in clinical trials as a potential screening tool for the detection of PE. Elevated sFlt-1/PlGF ratios have been observed in early-onset PE[19,20]. Chappell *et al*[19] published data from a multicentre trial using a PlGF assay (Triage, Alere, California, United States). PlGF concentrations below the 5th centile had a sensitivity of 96% and a negative predictive value of 98% for PE requiring delivery within the next fourteen days, in gestational ages below 35 wk. Beyond 35 wk, the test was not as good at excluding PE. This further strengthens the argument for a role of placental insufficiency in early cases of PE, with a weaker association between PlGF and late-onset PE.

PATHOLOGICAL EVIDENCE FOR THE AETIOLOGY OF PE

Many scientific evaluations of the pathology associated with PE have been used to justify the placental origins hypothesis of PE. These may be genetic, molecular or histological in nature.

Genetic

There is an unquestionable link between the development of PE and subsequent cardiovascular disease. Despite the incomplete understanding of the aetiology

of PE, familial clustering has been observed[27,28] and reported in PE and therefore supports a genetic link. Many susceptibility genes for PE have been reported in the literature - the function of the majority of these loci remain unknown. The very few PE genetic loci with known associations have previously been implicated in adult cardiovascular disease. Despite further work in this field being required, it does seem entirely plausible that due to the similarities between PE and cardiovascular disease with clinical risk factors and postpartum cardiovascular legacy, there is indeed a shared genetic link between PE and cardiovascular disease.

Molecular

Recently published data on the molecular biology, also provide insightful evidence. Yung *et al*[29] investigated whether there was molecular evidence of a difference in placental stress response between cases of early and late PE. Investigating multiple stress-signalling pathways, the investigators demonstrated that activation of stress-signalling pathways was negatively correlated with gestational age, with a clear inflection for placentae beyond 34 wk gestation. Activation of these pathways was significantly higher in early PE than in late PE or controls. The authors reported no difference between placentas in late PE and normotensive controls. It appears that this group of investigators have provided the first molecular evidence of placental stress response in early PE with a lack of such a response in cases of late PE. This supports a placental cause for early PE, but further suggests that placental dysfunction may not necessarily be implicated in late onset PE.

Histological

Histopathological studies of placentae have also reported a difference between early and late PE[30-32]. Whilst in the majority of cases of PE the placentae are histologically normal, it is also found that histopathological lesions are mainly seen in preterm PE[22]. Pathak *et al*[33] carried out a blinded analysis and reported that placental hypoperfusion in the form of massive perivillous fibrin deposition was much more frequent in those with PET (OR 20.2) and SGA (8.9). However we acknowledge that this was in a small sample of pathological cases. Ogge *et al*[32] demonstrated the prevalence of placental hypoperfusion lesions were greatest in cases of early-onset PE (58%), as compared to 33% in late onset PE and 16% in term controls.

An interesting, recent study investigated placental perfusion using magnetic resonance imaging in early and late PE[34]. The study reports that women with early PE did indeed show smaller placental perfusion fractions when compared to controls. Interestingly, women with late onset PE had a larger placental perfusion fraction when compared to matched controls for gestational age. The increased placental perfusion fraction seen in late PE further supports the argument that late-onset PE is associated with larger placentae and babies, and therefore is unlikely to be due to placental insufficiency.

PATHOPHYSIOLOGY - THE ROLE OF THE CARDIOVASCULAR SYSTEM

The maternal cardiovascular system undergoes a series of changes in pregnancy, all of which have a role in ensuring adequate uterine perfusion, oxygen delivery and provision of nutrients to meet the demands of the growing fetus. These adaptations enable the pregnancy and the growth of the fetus to continue unimpaired. They include a rise in cardiac output, heart rate and plasma volume and a concomitant fall in total vascular resistance (TVR).

Despite a significant volume of literature regarding placental dysfunction in PE, data regarding the cardiac changes associated with PE are more scant, and also more controversial. The conventional belief was that early-onset PE is associated with reduced cardiac output and increased total vascular resistance, with maternal cardiac function succumbing early in the disease process. With regard to late-onset PE, the original data implied that this was a condition of raised cardiac output and reduced total vascular resistance, however this model has not been reported consistently[35,36]. The discrepancies reported in the cardiovascular profiles in women who present with PE can be attributed to certain confounding factors - antihypertensive medication, co-morbidities, different gestational ages and stages of labour.

Another significant reason for the discrepancies reported in the literature with regards to haemodynamics in PE is the use of cardiac output instead of the corrected index for body surface area, cardiac index. Cardiac index (CI) represents the cardiac output per square meter of body surface area. We believe that using this corrected index is superior to using cardiac output, which does not factor in height or weight of the subject. As human beings come in different shapes and sizes, with varied metabolic demands, we feel that comparison of cardiac output without correcting for body surface area is both inadequate and inaccurate.

Prior to the onset of symptoms of early-onset PE, there is a shift towards a reduced cardiac index in conjunction with a raised total vascular resistance, increased mean arterial pressure, contracted intravascular volume and reduced venous reserve capacity[37-42]. These findings suggest that the high resistance/low volume haemodynamic profile observed in women destined to develop early-onset PE is observed in the latent phase of the disease at mid-gestation[39,40].

In a study by Melchiorre *et al*[39] women who subsequently developed late-onset PE presented with raised total vascular resistance, but importantly, there was no difference in cardiac index between late-onset

PE and control groups. Whilst these findings have not been reported consistently in the literature, it is worth noting that in studies that did not corroborate the findings observed by Melchiorre *et al*[41], corrected cardiac indices were not employed.

In preeclamptics medicated with anti-hypertensives, the CI normalises and is comparable to CI in normotensive controls. The observed reduction in arterial compliance, has also been shown to be corrected following antihypertensive treatment in patients with PE[42].

Although some of the initial work into cardiac changes in PE showed conflicting results, more recent work has demonstrated a more consistent collection of findings. This is in part due to improved and newer techniques such as colour tissue Doppler and 2-dimensional speckle tracking derived strain and strain rate imaging, which are able to detect early and subtle changes in myocardial function in an objective manner, when used in conjunction with validated diagnostic algorithms.

Prior to the onset of clinical symptoms in women who develop early onset PE, there is an abnormal remodelling of the left ventricle which consists of concentric remodelling and hypertrophy[39,41]. Moreover in this group of women, there is evidence of mild diastolic dysfunction as well as impaired myocardial relaxation[39,41]. This impaired diastolic function is thought to be associated with the increased cardiac afterload (increased TVR) and abnormal left ventricular remodelling[39,41]. The abnormal pattern of remodelling observed in PE is similar to that observed in non-pregnant individuals with essential hypertension and is consistent with an impairment that is afterload-induced[39,41,43].

Studies assessing early myocardial changes, have reported that mild-moderate isolated left ventricular diastolic dysfunction is seen in approximately half of women with early onset PE[39,41,43] with one in five women having biventricular systolic dysfunction with associated left ventricular hypertrophy[41,43]. Melchiorre *et al*[39,41] have also demonstrated impairment in myocardial contractility in early onset PE using colour tissue Doppler and 2-D speckle tracking. Left atrial remodelling in PE has also been reported. These findings suggest that the pregnant heart in PE is working at its maximum potential capacity, and any additional stress could result in a deterioration of cardiovascular function.

Other authors have suggested raised intra-abdominal pressure (IAP) as a major aetiological factor to the development of PE[44,45]. They hypothesize that women with raised IAP will have compromised venous return to the heart, which will result in decreased blood flow in the vascular beds of the placenta, uterus, kidneys and liver. The result of this impaired blood flow and venous congestion would include placental ischaemia, oedema of the lower extremities, glomerulopathy associated with hypertension and proteinuria and hepatic dysfunction[44,46]. This hypothesis is in concordance with the cardiovascular origin of PE proposed in this review. The myocardial dysfunction of PE noted in many studies will also compound the compromised venous return to the heart. These two theories are therefore not mutually exclusive.

WHAT IS THE CARDIAC LEGACY OF HAVING PE?

Although we have classically believed that treatment of PE is by delivery of the placenta in order to avoid impending maternal harm, the subtle, subclinical cardiac changes associated with a diagnosis of PE are not cured by birth of the infant or delivery of the placenta. Melchiorre *et al*[47] showed that at 1 year postpartum 56% of women with early onset PE had asymptomatic left ventricular dysfunction compared to 14% of women with late onset PE and 8% of healthy controls. The same authors also reported a 40% incidence of essential hypertension at 2 years post-delivery.

Indeed a diagnosis of PE is considered a risk factor for long term cardiovascular disease[48,49]. Following a diagnosis of PE, in particular early onset PE, it therefore seems prudent that these patients are adequately risk assessed in the years following pregnancy, in order to address and mitigate future cardiovascular risks.

CONCLUSION

Herein we argue that in order for PE to manifest in an expectant mother, there is an element of both a dysfunctional placenta as well as an abnormal cardiac response. Figure 1 depicts the common and specific key components of impaired placentation and cardiovascular dysfunction. We cannot ignore the role of the maternal cardiovascular system in the development of PE, and thus PE must be recognised and managed, both in the acute and in the long term phases, as a cardiovascular-placental syndrome.

PE is a heterogenous disorder. There is an abundance of evidence supporting the role of the placenta in PE. The evidence indicates that placental dysfunction is prevalent in early-onset PE, however this is not as clear in cases of late-onset PE, as evidenced by uterine artery Doppler indices, birthweight distribution, placental biomarkers, histological and pathological studies. Table 1 summarises the main differences in placental and cardiovascular parameters observed in early-onset vs late-onset pre-eclampsia

The majority of PE is late-onset, and has classically been described as "maternal" or "heterogenous" PE. By and large, maternal PE remains unexplained. Several theories involving an exaggerated maternal systemic inflammatory response and increased systemic levels of oxidative stress have been published in the past[50].

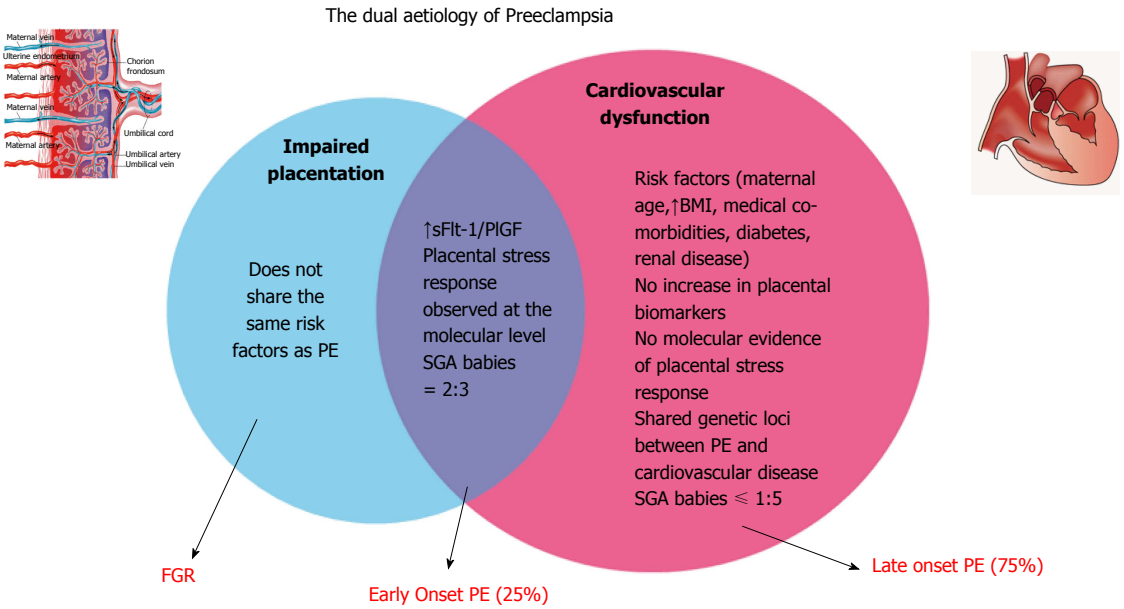

Figure 1 Illustration of the key components, both specific and common to impaired placentation and cardiovascular dysfunction and their presence in fetal growth restriction, early-onset and late-onset preeclampsia. FGR: Fetal growth restriction; PE: Preeclampsia; PlGF: Placental growth factor; sFlt-1: Soluble fms-like tyrosine kinase-1; SGA: Small-for-gestational-age; BMI: Body mass index.

Table 1 A summary of the key differences observed in placental and cardiovascular parameters in early-onset *vs* late-onset preeclampsia

	Early onset PE	Late onset PE
Fetal growth restriction	↑↑↑	↑
Hormones - PlGF	↓	↔
Hormones - sFlt-1	↑	↔
Hormones - PAPP-A	↓	↔
Uterine artery Doppler resistance	↑↑↑	↑/↔
Molecular stress response	↑	↔
Histological evidence of hypoperfusion	↑↑	↑/↔
Haemodynamics - Cardiac index	↓	↔
Haemodynamics - TVR index	↑↑	↑
Left Ventricular geometry – concentric remodelling	↑↑	↑↑
Left Ventricular geometry – concentric hypertrophy	↑	↓
Chamber diastolic function	↓↓	↓
Chamber systolic function	↓	↔

PE: Preeclampsia; PlGF: Placental growth factor; sFlt-1: Soluble fms-like tyrosine kinase-1; PAPP-A: Pregnancy associated plasma protein A; TVR: Total vascular resistance.

Whilst this is supported by the observed increase in PE in women with certain pro-inflammatory systemic conditions (autoimmune disease, renal disease, *etc.*)[51], another, and we believe stronger, argument can be made for the role of the maternal cardiovascular system in the development of PE.

PE is a complex disorder which is no doubt closely related to placental insufficiency. However we strongly believe that the placenta is not the only culprit; the inability of the maternal heart to adapt to placental dysfunction also forms a significant, however as yet incompletely understood, part of the enigma.

We propose that whilst intrinsic placental dysfunction and the mal-adaptation of the maternal cardiovascular system leads to early-onset PE, late PE is associated with an acquired placental dysfunction as a result of the maternal heart not being able to meet the demands of the placenta. Both the intrinsic and acquired placental dysfunction results in placental hypoxia which sets off a cascade of events result in the multisystem disorder of PE. In view of the evidence, we propose a paradigm shift of our current understanding of pre-eclampsia, as a disease entity not solely due to the placenta, but as a cardiovascular-placental syndrome. In answer to our original question which forms the title of this review, the answer is both - as an element of dysfunction in both the cardiovascular system and the placenta are required in order for PE to develop.

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MINIREVIEWS

Universal screening for hemoglobinopathies in today's multi-ethnic societies: How and when

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Abstract

Increasing multi-ethnicity in countries endemic or non-endemic for hemoglobinopathies has brought fundamental changes to the screening strategies for these traits. While in the past pre-screening on microcytosis was a reasonable method to economize upon follow up analysis, selecting low mean corpuscular volume means today missing all those normocytic carriers of common traits associated with severe conditions. Therefore, blood count should not be considered as a pre-selection tool but as additional information to be used for the interpretation of the provisional results, obtained by routine high throughput separation and measurement of the hemoglobin (Hb) fractions. Moreover, the moment of screening should be well planned depending on the social and cultural situation. Screening for genetic diseases in a modern multi-ethnic society should be offered to couples seeking progeny when both partners are more likely to be equally concerned with the good health of their children. In several societies screening before marriage and changing partner choice is culturally accepted. However, new generations are bound to disagree with these more or less imposed conditions and may decide not to renounce the choice of their partner asking for other preventive methods. In addition, a carrier state during pre-marital screening may in some cultures stigmatize the carrier, mostly the female with adverse social consequences. Therefore, screening for hemoglobinopathies early in pregnancy is the most sensible alternative in modern countries. Adding hemoglobinopathies to the routine rhesus screening using a simple separation of the Hb fractions on dedicated devices (high performance liquid chromatography or capillary electrophoresis) will virtually identify all female carriers of all common traits responsible for the severe conditions mainly sickle cell disease and thalassemia major in time for partner analysis, counseling and primary prevention.

Key words: Sickle cell disease; Thalassemia; Diagnosis; Prevention

Core tip: All women in most modern countries are offered screening for rhesus antagonism and infectious diseases early in pregnancy. Hemoglobinopathy (HBP) screening done together with rhesus screening using an inexpensive routine high performance liquid chromatography or capillary electrophoresis analysis could identify all women carriers of the frequent traits associated with the severe forms of HBP. Subsequent partner analysis could identify all couples at risk to be confirmed by molecular analysis in time for prenatal diagnosis when requested. This procedure will allow the prevention task to be offered at the most logical moment and by the most specialized hands of the gynecologists and obstetricians in collaboration with the local labs and the genetic counselors.

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INTRODUCTION

While up to a recent past were the Mediterranean countries those concerned with endemic hemoglobinopathy (HBP), with the thalassemia syndromes being the almost exclusive traits to be identified, today both Southern and Northern Europe are becoming a melting pot of different cultures from Africa, Middle and Far East, with different HBP traits to be identified[1]. In fact the same ethnic changes are observed all over the world and without prevention strategies many immigration countries will be confronted with a dramatic increases of severe HBP leading to human suffering and to increased costs of public health for the management of these incurable and expensive diseases[2,3]. To prevent all this, a broader spectrum screening for relevant HBP would have to be done in the endemic countries of Southern Europe and new national screening and managing strategies would have to be introduced in all other non-endemic countries experiencing massive immigration[4].

Over the past decades, regular beta thalassemia trait screening was based upon pre-selection of healthy but slightly anemic and microcytic carriers by measuring their low mean corpuscular volume (MCV) eventually persisting after iron therapy. This pre-selective screening method was considered practical and economically justifiable due to the fact that a complete blood count (CBC) was less expensive than screening by hemoglobin (Hb) electrophoresis and manual measurement of the HbA2 fraction[5]. This strategy might still be practical for large population screening campaigns for thalassemia in emerging countries. However, for screening and regular diagnostics in (non-endemic) developed countries this pre-selective strategy is inefficient and not cheaper at all.

Table 1 Cross table indicating the risk for the progeny of carriers of the common β globin gene defects

β traits	β minor	HbS	HbE	HbC	HbD	HbY
β minor	β major					
HbS	SCD	SCD				
HbE	β major	SCD	β minor			
HbC	β minor?	SCD	β minor	β minor		
HbD	β minor	SCD	β minor	Normal	Normal	
HbX	?	?	?	?	?	?

Hb: Hemoglobin; SCD: Sickle cell disease; HbD: HbD^{Punjab}.

In fact, even if well applied, this procedure implies at least two visits of the anemic carrier to the general practitioner (GP), two CBC's and at least one iron prescription. Moreover, healthy but slightly anemic thalassemia carriers are often kept on iron therapy for a long time before the hemoglobinopathy option is considered and investigated. In this scenario, delayed diagnosis of beta thalassemia carrier status in couples at risk might easily happen, resulting in the birth of a first severely affected child.

To avoid this unfortunate way of (mis) managing diagnostics and prevention, carrier screening campaigns at school, before obtaining the marriage certificate and before or early in pregnancy have been organized for several decades in most endemic Mediterranean countries, but often following the same strategy, being CBC as the first step followed by Hb separation and HbA2 measurement in case of low MCV[6-13].

Today these methods are inappropriate. Dedicated high performance liquid chromatography (HPLC) and/or capillary electrophoresis (CE) devices are available in all modern laboratories where separation and estimation of all normal and abnormal Hb fractions is obtained automatically (Figure 1). These automatic methods are much faster and cheaper to public health than the procedure mentioned above, involving GP visits, inadequate iron therapies and repetitions of non-conclusive laboratory analyses. Moreover, carriers of common traits like HbS and HbC or less common traits like HbD^{Punjab} or HbO^{Arab}, all associated with sickle cell disease (SCD), are not anemic or microcytic unless an independently inherited alpha thalassemia trait is also present. As a matter of fact, those few SCD carriers diagnosed today are identified just by chance, because of their border line MCV due to a coexisting alpha + thalassemia trait (-α/αα) or because of diabetes control with HbA1c later in life. Moreover, carriers of HbE, at risk for both severe beta thalassemia and SCD in their progeny, are often just border line microcytic, especially if vitamin B12 deficiency is also present. Therefore, pre-selecting by MCV in a multi-ethnic society, means missing the majority of the carriers of relevant traits associated with severe conditions in the progeny (Table 1). Screening should also not be based upon ethnic origin selection but should be offered at

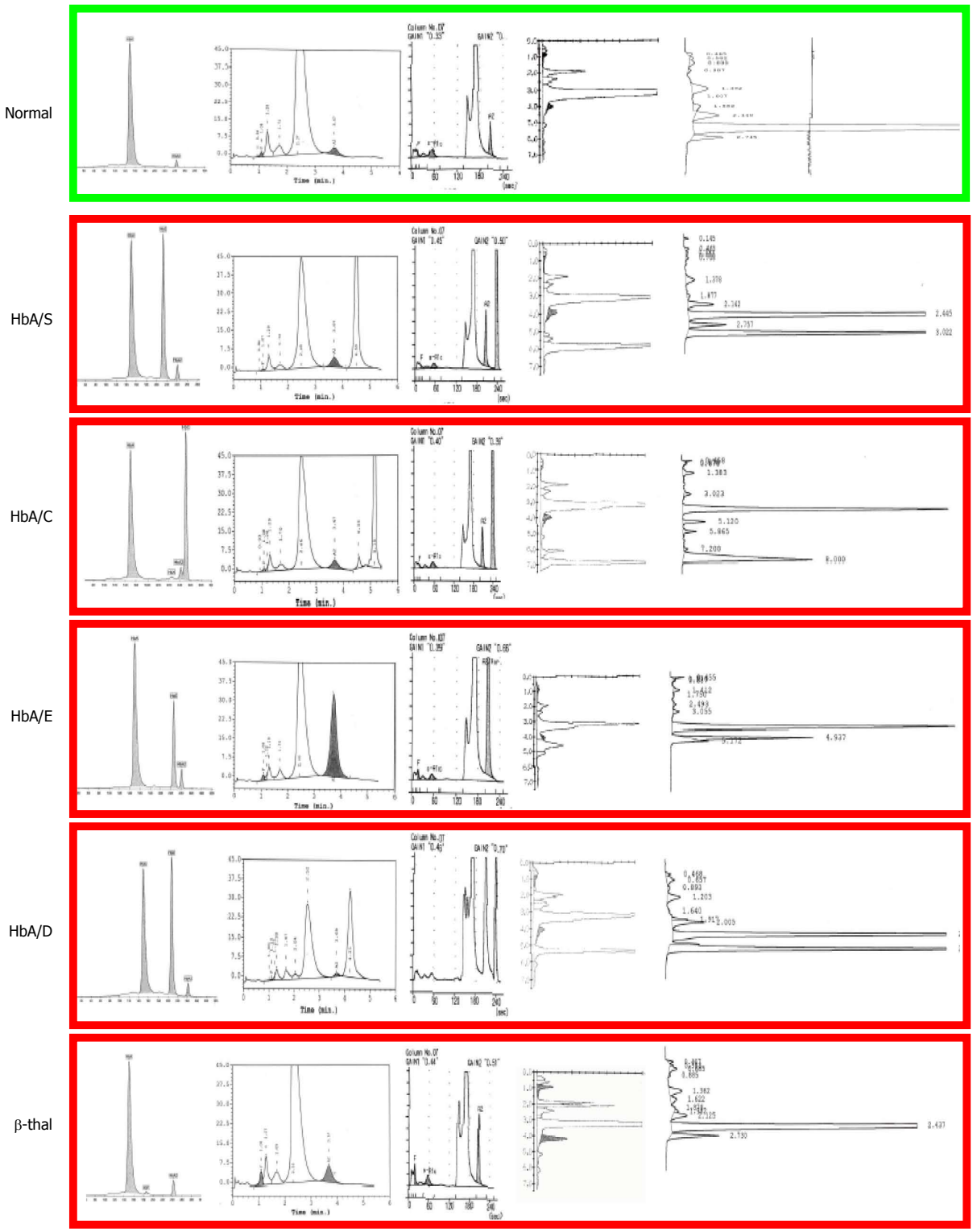

Figure 1 Examples of putative diagnostic results identifying carriers of the common β globin gene defects associated with sickle cell disease and β-thalassemia major on different high performance liquid chromatography or capillary electrophoresis devices. From left to right separations on Sebia CE and Bio-Rad, Menarini, Tosoh and Ultra2 HPLC devices. HPLC: High performance liquid chromatography; CE: Capillary electrophoresis; Hb: Hemoglobin.

the national level and at the most logical moment. Ethnic selection has been matter of discussion since HBP in some non-endemic European countries has been considered as a rare "import" disease, too infrequent in the general population to justify universal screening. Indeed HBP are not found very frequently in the so called "low risk" North-European populations but low risk is not "no risk" and carriers of beta thalassemia, alpha thalassemia and HbD^{Punjab} are regularly diagnosed in North-Europeans and not only because of distant (forgotten) "ethnic ancestors" but also due to "local" mutations. In fact, selecting for

ethnic origin means discriminating both the included and the excluded ones, and adds to the risk when having found a carrier in the high risk population, the low risk partners in mixed couples are not checked, just supposing that for them the chance of being a carrier is "very low".

Because of all these considerations newborn screening (NBS) is universal in most countries that offer this option. However, NBS is not providing primary prevention and not even retrospective primary prevention to couples at risk if they are not properly counseled[4,5,14].

For primary prevention, in the new multi-ethnic societies, screening should be offered to couples at risk before the first affected child is born and how and when universal screening should be organized starting with separation and measurement of the Hb fractions on HPLC or CE and using CBC as auxiliary data is further explained in this review.

The HBP's in a nutshell

To understand the HPLC/CE and CBC results one should keep in mind that thalassemias and abnormal hemoglobins are both HBP.

Thalassemias are caused by mutations on the globin genes that impair the expression of the genes reducing the amount of globin which is needed to form normal hemoglobin. This explains the microcytic hypochromic state of the red cells, the typical erythromorphology and the qualitatively normal HPLC/CE separations.

Abnormal hemoglobins (like the common HbS, HbC, HbE and HbD) are caused by mutations on the (beta) globin genes that change the structure of the globins and herewith of the more or less normally expressed abnormal hemoglobins. This explains the normocytic normochromic state of the red cells and the abnormal HPLC/CE separation. An exception among the common variants is HbE which is an abnormal hemoglobin with lower expression (approximately 25%) with a mild thalassemic phenotype in the carrier.

The genes involved in Hb expression during pre and postnatal life are the embryonic epsilon and zeta (ε and ζ), the fetal gamma (γ), the full expression beta (β) and alpha genes (α) and the low expression delta (δ) genes. All Hb molecules expressed during embryonic, fetal and postnatal life are tetramers. From the fetal life on, these tetramers are formed by 2 alpha and 2 non-alpha like globin chains. In normal conditions, the expression of postnatal hemoglobin HbA (α2/β2) and HbA2 (α2/δ2) is coded by two β-globin genes (one of paternal and one of maternal origin) located on chromosome 11, four α-genes (two of maternal and two of paternal origin) located on chromosome 16 and two δ genes, adjacent to the β-genes. The four γ genes needed for fetal HbF (α2/γ2) expression during fetal life, are silenced during the first year of life. Therefore the expected expression of the Hb fractions in normal adults will be approximately 96%-97% HbA 2.5%-3% HbA2 and 0.5%-1% HbF. All changes found in these parameters can be associated with a relevant hemoglobinopathy.

METHODS: SCREENING HOW

The samples

Materials and methods for "basic" HBP carrier screening are very simple and available in virtually all laboratories[15]. Screening should always include separation and estimation of the Hb fractions on automated HPLC or CE and the separation results should be compared for compatibility with the CBC parameters. Samples should be collected in EDTA and be processed directly for the best CBC and Hb separation results. However, samples kept refrigerated and processed a few days later are still valid for separation and measurement of the fractions and for DNA extraction if required. Dry samples, as eventually used for newborn screening, are suitable for obtaining a provisional result from the separation on HPLC or CE but are obviously not suitable for CBC and DNA extraction from dry samples requires special methods.

Separation of the Hb fractions on dedicated devices

Separation and measurement of the Hb fraction has been done automatically for more than two decades using dedicated devices. The most popular HPLC and capillary electrophoresis devices (CE) evaluated by Van Delft *et al*[16] are available in virtually all laboratories.

These devices identify rapidly and at very low cost all carriers of high HbA2 beta thalassemia and of the common and relevant HbS, HbC, HbE and HbD which will represent most of the positive results to be expected in a multi-ethnic population[16].

HbA2 levels above 4% are diagnostic for beta thalassemia trait with HbF levels that may or may not be slightly elevated[15]. Some cases of beta thalassemia trait may however present with HbA2 levels lower than 4%. In these cases, if the CBC remains microcytic, alpha and beta thalassemia should be further investigated at the molecular level, especially when risk is suspected in a putative carrier couple. In some cases, interfering delta globin gene defects may cause low HbA2 (δ2/α2) levels and especially delta thalassemia may interfere with the diagnosis of high HbA2 beta thalassemia[17].

Carriers of the common Hb variants (HbS, HbC, HbE and HbD) are identified at 100% sensitivity and over 95% specificity (Figure 1)[16]. The high specificity is partially due to the precise positions in which these fractions migrate but mainly due to the fact that these variants are the most common. To exclude rare variants moving in the same position as HbS the fraction can be confirmed with 100% specificity using the sickle test[18]. For all presumed couples at risk confirmation should be performed at the molecular level. All rare variants not corresponding to the common one should also be investigated at the molecular level to exclude genetic risk.

Table 2 Genotype/phenotype correlation, indicative MCV/MCH values, HPLC/CE separations and presence of HbH or Hb Bart's and genetic risk in alpha thalassemia

Genotype	α-thalassemia Condition name	Phenotype	MCV MCH	HPLC CE	HbH (adult) Hb Bart's (newborn)	Genetic risk for the progeny of carriers
αα/αα	Normal	Normal	Normal	Normal	Absent Absent	None
-α/αα	α^+ heterozygous	Eventually anemic	borderline	Normal	Absent 0%-5%	HbH
-α/-α	α^+ homozygous	Mildly anemic	Low	Normal HbA2↓	Absent 5%-10%	HbH
--/αα[1]	α° heterozygous	Mildly anemic	Low	Normal HbA2↓	Absent 5%-10%	HbH or Hb Bart's HF
--/-α[2]	HbH disease	Intermediate hemolytic anemia	Lower	HbH Hb Bart's HbA2↓↓	0%-10% 10%-30%	HbH or Hb Bart's HF
--/--	Hb Bart's Hydrops Fetalis	Severe intrauterine hemolytic anemia	Severe morphology	No HbA or HbF in newborn	Hb Portland Hb Bart's	Lethal to newborn. Life threatening for mother

[1]Rare positive cells after inclusion bodies staining; [2]Many positive cells after inclusion bodies staining (Figure 2). MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; Hb: Hemoglobin; HPLC: High performance liquid chromatography; CE: Capillary electrophoresis.

Table 3 Indicative parameters usually associated with carrier states

Carrier of	Hb	MCV	MCH	RBC	HbA2	HbF	Approximately HbS (%)	Approximately HbC (%)	Approximately HbE (%)	Approximately HbD (%)	Smear
Alpha thal	L[2]	L[2]	L[2]	N	N[1]	N					TC, APC
Beta thal	L	L	L	N or E	E[4]	N or E					TC, APC
HbS	N[1]	N[1]	N[1]	N	N[3]	N	45-25[6]				Normal[5]
HbC	N[1]	N[1]	N[1]	N	N	N		45-25[6]			TC
HbE	L-N	L-N	L-N	N	N	N or E			± 25		TC, APC
HbD	N[1]	N[1]	N[1]	N	N	N				± 45	Normal

[1]Could be lower in case of alpha thal; [2]Low depending on the form of the defect (see Table 2); [3]Could be higher due to HPLC artifact; [4]Some rare beta thal carriers have normal HbA2; [5]Positive on sickle test; [6]%HbS or C reduced in the presence of alpha thal. L: Low; N: Normal; E: Elevated; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; RBC: Red blood cell; TC: Target cells; APC: Anisopoikilocytosis; Hb: Hemoglobin.

Results of HPLC/CE screening and prevention

Screening with HPLC/CE methods will practically identify all carriers of "beta gene defects" that are important for offering primary prevention of severe hemoglobinopathies in a multi-ethnic society. Adult carriers of mild alpha gene defects are not identified on HPLC/CE but can be easily identified during newborn screening by the presence of Hb Bart's (Table 2).

Due to the lethal outcome of homozygous alpha zero defects and the usually mild to intermediate phenotype of HbH disease, carriers of alpha gene defects represent a relatively less severe problem to public health. However, and also because of maternal risk, screening for these traits, common in Southeast Asian and Chinese populations, should be taken into serious consideration (read further).

Correlation between HPLC/CE and CBC parameters

The CBC parameters should be compatible with the HPLC or CE results. As mentioned above, carriers of thalassemia are characterized by a mild microcytic (MCV↓) hypochromic (MCH↓) anemia (Hb↓) not improving on iron therapy. Conversely, carriers of the common Hb variants, separable on HPLC or CE are not microcytic, unless also carriers of coexisting alpha thalassemia. In addition, a low MCV value tends to increase with time and in case of borderline values measured often in mild alpha thalassemia, one can better rely on the MCH value which remains more constant. The best discriminating CBC parameter to differentiate between iron deficiency and thalassemia will be the red blood cell (RBC) count, which is often elevated in the compensated beta thalassemia carrier with sufficient folic acid intake. Indicative parameters are summarized in Table 3.

The microcytic anemic condition is less evident in carriers of mild alpha thalassemia. This depends from the presence of 4 alpha genes and from the number of alpha genes left active. CBC can then be nearly normal in the mildest alpha thalassemia forms and strongly abnormal in the severe HbH disease form (Table 2).

Erythromorphology

While often disregarded in modern laboratories because time consuming and because it requires trained observers, the smear gives away the diagnosis of thalassemia to the expert eye who will identify the typical erythromorphology of the target cells and anisopoikylocytosis (last column in Table 3), the last being the visual equivalent of the differential

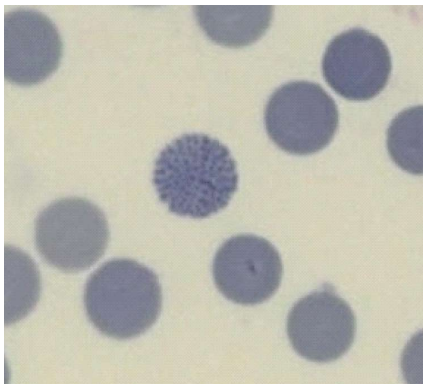

Figure 2 Typical hemoglobin H inclusion bodies found sporadically in carriers of alpha zero thalassemia (--/αα) and abundantly in patients with hemoglobin H disease (--/-α).

parameter "red cell distribution width" (RDW). Moreover, smears stained with supravital brilliant cresyl blue (reticulocytes staining), will identify the rare HbH inclusion body cells, specific to the alpha zero thalassemia carrier (at risk for hydrops fetalis in the progeny) (Figure 2).

Significance of HbA2 and HbF measurement

As mentioned above, an adult β-thalassemia carrier will usually show 4% or more HbA2. Then, if matching with the expected microcytic hypochromic CBC parameters, the provisional diagnosis will be "carrier of β-thalassemia". However, some rare β-thalassemia mutations show HbA2 values in the "grey zone" (3.5%-3.9%) or even normal levels. In these cases excluding a β-thalassemia trait because of a value just below the normal cut of, trusting on the presumed precision of the measurement is very imprudent, especially when CBC indicates persistent microcytosis in absence of iron depletion. In these cases further investigation is needed as one may have a borderline HbA2 β-thalassemia, an α-thalassemia or a large β deletion thalassemia defect[19]. Some HbF (up to approximately 7%-8%) will eventually be present in the beta thalassemia carrier. Higher HbF levels are usually associated with delta/beta thalassemia deletion defects that will present with normal HbA2 levels and masked microcytosis due to the larger size of the HbF cells.

Dedicated devices may overestimate or underestimate the HbA2 levels in the presence of Hb variants. However, measurement of HbA2 in the presence of HbA and any β variant is obsolete. When both HbA and the β variants are expressed (in non-transfused patients) it means that both β genes are expressed and that there is no need to measure HbA2 to establish the presence of a non-expressed β thalassemia gene.

HbA2 and alpha thalassemia

Adult α-thal carriers will show no specific characteristics on HPLC or CE except for a marginal reduction in HbA2 expression. The only exception is HbH disease, which will usually present with significantly low HbA2 and a rapid migrating or eluting but unstable and quickly disappearing HbH fraction.

When suspecting α-thal because of the CBC parameters in the absence of iron depletion and low HbA2, then molecular analysis is needed to detect the common deletions or point mutations. On the other hand, α-thal can easily be detected at birth by the presence of Hb Bart's. The presence of Hb Bart's observed during newborn screening should be reported to avoid a more laborious diagnosis later in life.

In the presence of a single non active α gene Hb Bart's value between 0% to 5% is usually measured. The rates rises from 5% to 10% when two α genes are compromised (either in *cis* --/αα or *trans* -α/-α), while in HbH disease (-α/--), Hb Bart's level can go from 10% to 30%. In case of the lethal hydrops fetalis syndrome (--/--) no HbF is present in the fetus or in the severely compromised newborn but traces of HbH (β4), Hb Bart's (γ4), (ε4), Hb Gower-I (ζ2ε2) and the embryonic Hb Portland (ζ2γ2) are detected (Table 2).

The folic acid issue

Together with iron are vitamin B12 and folic acid essential for erythropoiesis. Folic acid is prescribed before and during pregnancy also to prevent neural tube defects like spina bifida. Folic acid is a vitamin with limited body storage that can be rapidly exhausted during increased cell division, a condition not only present in pregnancy but also chronic in thalassemia carriers. As mentioned above, the RBC count tends to be elevated in well compensated beta thalassemia carriers. Compensation is folic acid dependent and anemia may become more pronounced in beta thalassemia carriers in case of low folic acid intake. If folic acid is sufficiently present, RBC counts tend to be higher and by this compensatory mechanism Hb levels tend to be higher as well, while the packed red cells (PCV) value remains within normal levels. Measurement of serum folic acid gives no clue since it is the limited reserve of this vitamin which determines maintenance of the compensating mechanism. Therefore a folic acid prescription for anemic thalassemia carriers with poor dietary intake can be beneficial.

SCREENING WHEN

Newborn screening

Although SCD and thalassemia major do not need special treatment during the first 5-6 mo of life, several immigration countries have included HBP in the ongoing newborn screening (NBS) originally intended for treatment of metabolic diseases directly after birth[20-24]. Although NBS comes one child too late, one could expect some retrospective and prospective primary prevention for couples who had an affected or a carrier child. Unfortunately, after 7 years of NBS in the Netherlands no changes in the incidence of these severe diseases and in the requests for prenatal

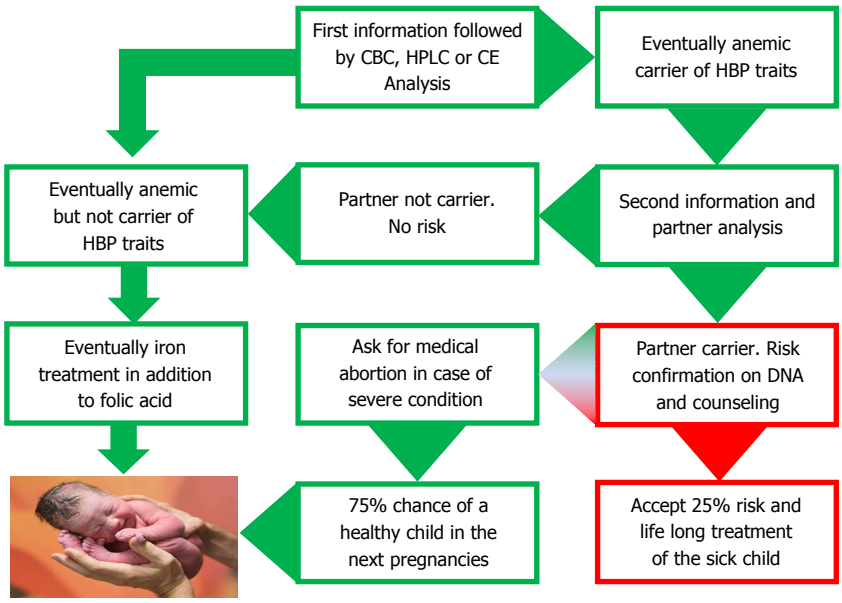

Figure 3 Genetic risk for hemoglobinopathies and practical prevention flow chart after carrier screening early in pregnancy. HPLC: High performance liquid chromatography; CE: Capillary electrophoresis; HBP: Hemoglobinopathy; CBC: Complete blood count.

diagnoses have been registered[14].

More efficient screening

To introduce a more efficient screening for primary prevention in multi-ethnic societies, one needs to identify healthy couples at risk before the birth of the first affected child. Moreover, one needs to choose the most logical moment, to provide the most consistent structure and to apply the most economically and socially convenient strategy[24]. If we look at the experience from decades of prevention in endemic countries[7-13], we see mandatory screening options before marriage which are not likely to become recommended in modern multi-ethnic societies unless prescribed by specific cultures. Screening when planning a pregnancy would be ideal but no structures are available to deal with different cultures at this stage except perhaps the scarcely experienced and reluctant GP's. In fact, pre-pregnancy counseling is not available in most of the non-endemic immigration areas where little awareness is present in the daily healthcare structures and in the public. Putting up such a structure would take lot of efforts, lot of time and money. Regarding PGD, most countries offer this only in case of fertility problems and/or embryo selection when a suitable stem cells donor is needed for an affected child.

Screening early in pregnancy has been proven to be most effective and requested by well-informed couples in the endemic countries of Southern Europe and it is bound to be the most sensible options in most immigration countries (Figure 3). Screening early in pregnancy comes at a time when both parents are concerned with the good health of their progeny, when they are both generally involved and when the female partner has lesser chances to be stigmatized. Moreover, pregnancy care structures are usually well organized in most immigration countries where dedicated midwifes and gynecologists can provide information and offer screening and prevention[25]. The most practical option would then be to anticipate the first pregnancy visit and to offer hemoglobinopathy screening together with the rhesus and infectious diseases screening which is routinely offered to all pregnant women in most developed countries.

Screening results: Information vs counseling

An important task for the obstetrician/gynecologist and for the laboratory analyzing the blood is to provide a thorough explanation. This can be done verbally and by using a standard letter when offering screening. Once the (pregnant) female carrier is diagnosed, short term partner analysis should be advised and to avoid anxiety it should be explained that being a carrier is not a disease but a recessive hereditary trait that only if present in both parents may affect their children with a chance of 1 in 4 causing a severe condition. Couples at risk should be made aware of the fact that the risk of 1 on 4 goes for every pregnancy and that having had one affected child does not mean that the next 3 should be healthy. Carriers should be reassured by explaining that each human is a carrier of several recessive traits and that knowing which one is an advantage that allows prevention if necessary.

Once the partner of a carrier of a relevant trait is found positive, then the putative couples at risk should be confirmed at the molecular level and counseled by a genetic counselor well informed about the severity of the diseases and the prognosis to be expected in the progeny. If the parents opt for prevention, prenatal diagnosis should be provided. Counseling should not be directive, neither toward treatment nor prevention,

but based upon thorough and realistic information and should not be provided by doctors that may propose very expensive treatment without any hope of a definitive cure[3]. Parents should be counseled about the difference between treatment and cure. They should be made aware of the fact that the disease can be treated but cannot be cured and that, in spite of the best treatment, the severity of the disease will only progress until premature death. Bone marrow (stem cells) transplant (BMT) the only available "cure" today, may also fail causing severe graft versus host diseases and even when "successful" may leave the patient in a chronic post BMT condition requiring life-long treatment and monitoring.

Some bottlenecks

Information on genetic risk can be interpreted in different ways in different cultures. In some cultures, when the pregnant female is diagnosed as a carrier and the male partner is asked to be checked, he might refuse to comply. In some culture the male might blame the female for getting a sick child. There have been cases of pregnant HbS carriers who had a child affected with SCD because the father did not show up, leaving the mother alone to take care of the sick child. It would therefore be ethically correct to offer prenatal diagnosis to female carriers also in absence of the father and in particular when the supposed father belongs to an ethnic group of high prevalence.

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MINIREVIEWS

Emergency contraception: What is new?

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Abstract

Unintended pregnancy rates remain high throughout the World and increase the risk of poor maternal and infant outcomes. Most of unintended pregnancies occur in women who were not using contraception or who became pregnant despite the reported use of contraception. Women who have had recent unprotected intercourse including those who have had another form of contraception fail are potential candidates for this intervention. Currently used emergency contraceptive methods are pills that contain combined estrogen-progesterone, only progestin, antiprogestins and copper intrauterine devices. The most common form of this type of contraception is oral progestin-only pills (levonorgestrel). The most effective method is copper intrauterine devices followed by anti-progestins and oral progestin-only pills. The major pathogenesis of oral emergency contraceptives is the prevention or delay of ovulation. Although conception is possible on only a few days of the cycle, emergency contraception is offered when indicated without regard to the timing of the menstrual cycle because of uncertainty in the timing of the ovulation. Levonorgestrel and E/P regimes are most effective as soon as possible after unprotected sexual intercourse. A linear relationship has been shown between effectiveness and the time of dose. The effectiveness continues for 120 h, but it is recommended to be used within 72 h after intercourse. Intrauterine devices may prevent pregnancy when 5 d after ovulation.

Key words: Emergency contraception; Levonorgestrel; Mifepristone; Ovulation; Ulipristal acetate

Core tip: Emergency contraception methods have varying ranges of effectiveness depending on the method and timing of administration. The major pathogenesis of oral emergency contraceptives is the prevention or delay of ovulation or prevention of fertilisation. Combined and progestin-based emergency contraceptives should be used as soon as possible to enhance the efficacy. Emergency contraception offers a final chance to prevent pregnancy.

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INTRODUCTION

Emergency contraception is defined as contraceptive methods used after unprotected sexual intercourse or sexual assault and in cases with contraceptive failure. Currently used emergency contraceptive methods are pills that contain combined estrogen-progesterone, only progestin, antiprogestins and copper intrauterine devices.

The most common form of this type of contraception is progestin-only pills (levonorgestrel). The most effective method is copper intrauterine devices followed by anti-progestins and oral progestin-only pills. The major pathogenesis of oral emergency contraceptives are presumed to be a delay or prevention of ovulation. Levonorgestrel in particular is ineffective as emergency contraception after ovulation. The major role of copper-induced intrauterine devices in emergency contraception is the prevention of fertilization[1]. There is much evidence suggesting that implantation of the fertilized ovule cannot be prevented by emergency contraception.

The possibility of pregnancy after unprotected sexual intercourse changes between 12%-30% in a population of young couples in their mid-twenties depending on the day of the menstrual cycle[2]. While the possibility of pregnancy is higher on the day of ovulation, emergency contraception can be used at any time of the menstrual cycle. It is independent of the day of ovulation. The most widely used EC methods are summarised in Table 1.

Indications for possible emergency contraception fallow as[3]: When no contraceptive was used during sexual intercourse within the previous 120 h; When there is a contraceptive failure or incorrect use of a contraceptive within the previous 120 h, including: (1) condom breakage, slippage, or incorrect use; (2) three or more 30 to 35 mcg ethinyl estradiol pills have been missed (or two or more 20 to 25 mcg pills); (3) progestin-only pill (minipill) taken more than three hours late; (4) more than two weeks late for injection of depot-medroxyprogesterone acetate; (5) dislodgment, breakage, tearing, or early removal of a diaphragm or cervical cap; (6) dislodgment, delay in placing, or early removal of a contraceptive hormonal skin patch or vaginal ring; (7) failed coitus interruptus (*e.g.*, ejaculation in vagina or on external genitalia); (8) failure of a spermicide tablet or film to melt before sexual intercourse; (9) miscalculation of the periodic abstinence method or failure to abstain on fertile day of cycle; and (10) expulsion of intrauterine contraception.

Table 1 Summary of emergency contrception methods

Contraceptive methods	Mechanism of action	Effective time	Most common side effects
Contraceptive Pills (Yuzpe Regimen)	Inhibition or delay of ovulation	72 h recommended (Up to 120 h)	Same as COC
Progestin-only ECP	Inhibition or delay of ovulation	72 h recommended (Up to 120 h)	Nausea and vomiting Irregular bleeding
Ullipristal	Inhibition or delay of ovulation	Up to 120 h	Nausea and vomiting Abdominal pain
Copper induced IUD	Prevention of İmplantation	Up to 5 d (recommended)	Genital infection Bleeding with unknown etiology Abnormalities of the uterus Wilson disease

ECP: Emergency contraceptive pills; IUD: Intrauterine devices; COC: Combined oral contraceptives.

COMBINED EMERGENCY CONTRACEPTIVE PILLS (YUZPE REGIMEN)

Many clinical studies have shown that ovulation can be prevented or delayed with emergency contraceptive pills that contain estrogen (E) and progesterone (P)[4,5]. After the first studies showing prevention of pregnancy with high dose of estrogen in 1974, Yuzpe *et al*[6] defined a low dose regime containing 200 mcg ethinyl estradiol and 1 mg levonorgestrel.

Some studies suggested that emergency contraceptive pills (ECP) prevent the implantation of the fertilized ovule by changing the endometrial receptivity in addition to the biochemical and histological changes in the endometrium after use of this regime. However, these suggestions were not confirmed in recent studies. Other possible mechanisms of ECP include changes to the function of the corpus luteum, thickening of cervical mucus, changes in tubal transport and prevention of fertilization[7-9].

Preven was approved for emergency contraception by Food and Drug Administration (FDA) in 1998 and was withdrawn in 2004. However, combined oral contraceptives (COC) that are not packed for emergency contraception can be used as 100 mcg ethinyl estradiol and 0.50 mg levonorgestrel with the same dose repeated in 12 h[10]. Levonorgestrel and E/P regimes are most effective after as soon as possible after unprotected sexual intercourse. There is a linear relationship between efficacy and the dose time. The efficacy continues for 120 h, but use within 72 h is recommended. Patients must be warned about this reduction in efficacy - especially after 96 h. In a meta-analysis of 3800 women, the success of the Yuzpe regime in the prevention of unwanted pregnancies was found to be 56%-89%[11]. However,

the Yuzpe regimen is not recommended because side effects are more common, and the efficacy of ECP is approximately half of the use of only levonorgestrel[12].

PROGESTIN-ONLY ECP

Early studies showed that levonorgestrel disrupts ovulation and the luteal functions. No effect on the endometrium was reported in these studies despite a study suggesting that the use of levonorgestrel glycodelin just before the luteinizing hormone (LH) surge changes the luteal phase secretory pattern. Levonorgestrel has no effect on the endometrial implantation of the embryo and endometrial receptivity markers[13-18]. The original prescription is the use of 0.75 mg oral levonorgestrel in the first 72 h with a repeated identical dose after 12 h. The same efficacy was reported with a 1.5 mg single dose in different studies. In addition to the studies supporting the same side effects for the two methods, most studies reported more headaches and breast sensitivity in the single dose regime[19,20]. In another study, 2 doses of 0.75 mg levonorgestrel repeated at 24 h had the same efficacy. Levonorgestrel is more effective than the Yuzpe regime in the prevention of pregnancy (RR = 0.51, 95%CI: 0.31-0.83) with fewer side effects (nausea RR = 0.43, 95%CI: 0.39-0.48; vomiting RR = 0.24, 95%CI: 0.18-0.31; headache RR = 0.83, 95%CI: 0.69-1.00; breast tenderness RR = 0.84, 95%CI: 0.69-1.01)[21].

ANTIPROGESTINS

Ullipristal and mifepristone are similar antiprogestins with similar chemical structures. The major mechanism is *via* prevention or delay of ovulation in addition to the effects on endometrium causing disruption of implantation[22].

MIFEPRISTONE

Mifepristone is a first-generation progesterone receptor modulator and is approved for use in many countries for first trimester abortion. In randomized studies, a dose of 5-600 mg mifepristone (RU-486) has the same or better efficacy in the prevention of pregnancy than oral emergency contraceptives (up to 99%-100%)[23-26].

The optimal dose is not clearly defined but may be 25-50 mg with no serious side effects. A delay in menstrual bleeding after the use of antiprogestins decreases doubts of pregnancy and anxiety[27,28]. The use of mifepristone in pill form is prevented for widespread use as emergency contraception. Mifepristone is only approved in Armenia, China, Russia and Vietnam as an emergency contraceptive.

ULLIPRISTAL

A selective progesterone receptor modulator with antiprogestin effects that can cause 5 d in delay of ovulation. There are studies showing that it can cause endometrial changes, however it remains controversial if it inhibits implantation. Ullipristal is used as a single dose of 30 mg. It is the most effective ECP in Europe and United States with an efficacy of 62%-85%[29-31]. Ullipristal is effective in the late follicular phase in which a rise in the level of LH has begun but no peak was achieved. In a meta-analysis comparing the efficiencies of levonorgestrel and ullipristal, the latter was shown to be higher at 0-24, 0-72 and 0-120 h after intercourse with no differences in side effect profiles[31].

COPPER-INDUCED INTRAUTERINE DEVICES

Implantation occurs 6-12 d following ovulation. Intrauterine devices may prevent pregnancy when applied 5 d after ovulation. Copper induced intrauterine devices (IUD) are the most effective emergency contraceptives with a major advantage for continuous contraceptive effect. In a multi-centre study with 1013 patients, its efficiency was 96.9% when used 120 h after intercourse[32]. The contraindications of applying an IUD at the same day of intercourse are acute cervicitis and other known medical contraindications. It should be applied 5 d after unprotected sexual intercourse, however there is limited data suggesting that the efficiency continues to 7 d[33].

OTHER INTRAUTERINE CONTRACEPTIVE METHODS

No data was found in the literature about the use of LNG-IUDs in emergency contraception. It was not recommended for this indication[34,35]. The LNG-IUDs can only be applied after the exclusion of pregnancy and only if the patient is 7 d or more into the menstruel cycle. If not, an additional contraceptive method or ECP for 7 d is recommended[35].

The frameless copper IUDs produced for adolescents and nullipar women (GyneFix® 330) have the same efficiency as ordinary copper IUDs in emergency contraception[36].

THE FACTORS CHANGING THE EFFICIENCY OF EMERGENCY CONTRACEPTIVES

Timing of treatment

Combined and progestin-based emergency contraceptives should be used as soon as possible (0-72 h) to enhance the efficacy[37]. Both drugs can be started up to 120 h after sex, but patients must be informed about the reduced activity after 96 h[38]. Ullipristal is the only approved oral agent for emergency contraception

from 72-120 h. Copper IUDs can be used for up to 5 d because of the post-fertilization effects. A systematic review reported the efficiency of IUDs up to 7 d, but there is limited data supporting this claim[39].

Body mass index

The efficiency of levonorgestrel ECP is decreased in obese women with an increase in unwanted pregnancies; however, no such effect was seen for ullipristal[39]. Because of these results, one commercial form of levonorgestrel added a warning of reduced efficiency in patients over 75 kg. After revision of the study, the European Medicines Agency described no reduction, and the warning was removed[40].

SAFETY

There is no physical examination or laboratory testing required before the use of oral progestin-based emergency contraceptives beyond the unnecessary pregnancy test unless there is no doubt of pregnancy due to symptoms or last menstruation date. No teratogenic effects on the foetus or side effects have been reported, however pregnancy must be excluded in cases using ullipristal or IUD.

Emergency contraception did not show any causal connection to death or serious complications. According to profit and loss rate data from the United States Medical Eligibility Criteria for Contraceptive Use (US MEC), no risk factors were reported to prevent the use of combined ECP and progestin-only pills[34,35]. ECP can be used in patients with lactation or a history of ectopic pregnancy, cardiovascular disease, migraine, and liver disease. ECPs can be used in patients contraindicated for combined oral contraceptives because of the short use time and lower total hormone dose[35]. Ullipristal acetate, progestin-only ECP, or copper-induced IUDs are preferred in women with changes in coagulation factors or a history of venous thromboembolism (VTE) or pulmonary embolism (PE). Repeated ECP use is safer than pregnancy even if there is no sufficient data for repeated ECP use. A warning to not repeat the dose in one menstrual cycle is included in the prospectus of ullipristal asetate[41].

CONTRAINDICATIONS

The contraindications for hormonal contraceptives cannot be adapted to women using emergency contraception. This is especially true in cases with cardiovascular diseases, thrombotic diseases, migraine, and liver disease. The advantages of use overpower the potential risks[42,43]. These guides do not include ullipristal. The contraindications for use of ullipristal are suspected pregnancy, uncontrolled asthma and liver diseases. The contraindications for IUD are uterine distortion, active pelvic infection, allergy to copper, and suspected pregnancy.

SIDE EFFECTS

Nausea-vomiting, abdominal pain, breast tenderness, headache, dizziness and weakness that regressed spontaneously in 24 h are the major side effects. Nausea and vomiting are seen in nearly half and 20% of the patients using combined ECP, respectively. Nausea and vomiting are less common in levonorgestrel than in combined ECP[12].

Some authors suggest a repeated dose in case of vomiting. This should be given 2 h later. In combined ECPs containing E/P, a repeated oral levonorgestrel and ullipristal dose is given at 1 h and 3 h after vomiting, respectively. Meclisine can be used to prevent nausea but it has no effect on dizziness. Vaginal administration is described, but the efficiency remains unclear. The application of the IUD is preferred instead.

ECPs containing levonorgestrel may shorten menstrual cycle when used in the early days of the cycle. ECPs have no effect on the duration of the cycle, but the duration of bleeding may extend to the next cycle. The duration of the menstrual cycle extends when used in periovulatory and postovulatory phases. Inter menstrual bleeding is seen in 15% of patients.

EFFECTS ON PREGNANCY

In a study of 332 pregnant women who used levonorgestrel in during the conception phase, there was no rise in birth defects[44]. ECPs do not increase the risk of extrauterine pregnancy in future pregnancies[45,46].

USE IN LACTATION

There is no restriction on the use of levonorgestrel or combined ECPs in lactating women[44]. In a study researching the pharmacokinetics of the use of 1.5 mg levonorgestrel for emergency contraception in lactating women, it is recommended to break from breastfeeding for 8 h during the excretion of levonorgestrel to breast milk. Feeding should restart in 24 h[47].

In a study comparing efficiencies of progestin-only oral contraceptives and progestin-only ECPs in lactating women, no difference was detected in maternal and foetal effects[48]. Seven days hiatus from breastfeeding is advised after a single dose of ullipristal. The milk must be collected and discarded during this time to continue to stimulate lactation[49].

DRUG INTERACTIONS

Drugs that induce liver enzymes may reduce the efficiency of levonorgestrel and ullipristal. Therefore, IUDs may be recommended for emergency contraception in patients using drugs inducing liver enzymes such as anti-epileptics and antiviral agents in the last 28 d[50]. In addition, ullipristal should not be

used with drugs increasing gastric pH.

PROCEEDING OR STARTING HORMONAL CONTRACEPTION

Because of the unknown duration of emergency contraceptive effects, it is unclear how to proceed with continuous contraception at the same menstrual cycle. The patient must be informed about using a safe contraceptive method[51]. According to pharmacokinetic studies and expert opinions, barrier methods and hormonal contraceptives may be started after application of emergency contraception. In the first seven days of hormonal contraceptives, an additional failsafe method must be used.

The use of long-term contraceptives is not recommended prior to exclusion of pregnancy. The efficiency of hormonal contraceptives may decrease in patients using ullipristal depending on the progesterone antagonistic effect. The FDA does not recommend the use of hormonal contraceptives within 5 d of ullipristal[52].

EXPERIMENTAL METHODS

Prostaglandin inhibitors

Cyclooxygenase enzyme (COX-1 and COX-2) inhibitors have female reproductive functions of oocyte maturation and ovulation. Many studies targeting this effect in emergency contraception are still underway. Studies have shown 15 mg of meloxicam with 1.5 mg levonorgestrel is more effective on follicular rupture in 5 d in patients with a follicular size over 15 mm than levonorgestrel only[53,54]. In addition, a 400 mg dose of celecoxib may delay or prevent luteal changes[55].

CONCLUSION

Emergency contraception is the last chance to prevent pregnancy after unprotected sexual intercourse or contraceptive failure. The efficiency increases by starting the medication as soon as possible. It is important to remember the rate of unsuccessful contraception and to exclude pregnancy especially in obese or drug-contraindicated patients. Of course, patients must be informed and encouraged to use regular and effective contraception.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Outcomes of surrogate pregnancies in California and hospital economics of surrogate maternity and newborn care

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Abstract

AIM: To describe maternity and newborn charges for an economic analysis of surrogate pregnancies on the health care resource utilization.

METHODS: A retrospective chart review of all women identified as being surrogates and the infants born from these pregnancies was performed between January 1, 2012 and December 31, 2013. Selected maternity diagnoses, mode of delivery, duration of hospitalization, and hospital charges were collected together with infants' birth weights, gestational age, length of hospital stay, and hospital charges. Charges associated with the *in vitro* fertilization cycles, artificial insemination, or embryo(s) transfer into the surrogate were not considered in the maternity charges. A ratio contrasting the maternity hospital charges for the surrogate carrier was compared as a ratio to the mean charges for 2540 infants delivered in 2013 after natural

conception and adjusted to the baseline hospital charges for both maternity and newborn care.

RESULTS: Analysis of sixty-nine infants delivered from both gestational and traditional surrogate women found an increased in multiple births, NICU admission, and length of stay with hospital charges several multiples beyond that of a term infant conceived naturally and provided care in our nursery. Among singletons and twins (per infant) hospital charges were increased 26 times ($P < 0.001$) and in triplets charges were increased 173 times ($P < 0.0001$) when compared to a term infant provided care in a normal nursery at our center.

CONCLUSION: Maternity costs for surrogates exceed those of women who conceive naturally, and these costs are especially magnified in women with triplets and multiple births.

Key words: Surrogacy pregnancy; Assisted reproductive technologies; Prematurity; Multiple gestations

Core tip: Surrogate pregnancies result in higher maternity and newborn costs with increased rates of multiple births and creates a moral hazard for hospitals. This increase occurs despite of the fact that surrogate mothers are prescreened for health and reproductive ability. Reduction in multiple embryo transfer would reduce the adverse economic impact of surrogate pregnancy, maternity and newborn costs.

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INTRODUCTION

In the United States approximately 7.4% of married couples are affected by infertility[1]. The causes of infertility are multiple and range from advanced maternal age, uterine malformation, hysterectomy, fallopian tube blockage, previous tubal ligation, lack of oocyte reserve in women, male factor infertility associated with oligospermia, pervious vasectomy with failed reconstruction, and other causes. In addition to fertility, in our evolving society where non-traditional family models are increasingly accepted, more and more single adults, or adults in same-sex relationships or marriage also desire to become parents and rear a family. In many such situations prospective parents may enter into an agreement to obtain oocytes or sperm, or use the surrogate's own egg and serve as a traditional surrogate for a pregnancy[2]. In other situations, a couple that has genetically related embryos created through *in vitro* fertilization (IVF) requires another women, a gestational carrier, in whom an embryo(s) and fetus(es) may develop. After birth, through a contractual relationship arranged prior to pregnancy, the gestational carrier relinquishes the infant(s) to the intended parents[2].

In many countries and in some United States states, traditional and gestational surrogacy is illegal. In the United States and its territories, a patchwork of laws regarding surrogacy exists[3]. Some United States states, limit the use of surrogacy, or permit surrogate pregnancies or use of gestational carriers only among married couples or the use of gametes from relatives, and in most states surrogacy contracts and their enforcement are determined by case law. Nevertheless, surrogacy is gaining greater societal acceptance in the United States. For instance, in California, one of the most liberal United States states in this respect, the law permits both traditional and gestational surrogacy in exchange for payment, and designates independent legal counsel for the surrogate and the intended parents, and the creation of a contract with judicial review and approval under the Uniform Parentage Act as amended in 2012[4]. However, the recruitment of women as traditional or as gestational surrogate carriers is unregulated in California. Further informed consent with thorough discussion of the risks associated with oocyte retrieval for some embryo transfers used in gestational surrogacy is unregulated in all states except California, and significant gaps have been identified in adherence to state statutes[5]. Despite the growing popularity of surrogacy, the medical complications associated with surrogacy and the related costs have not been precisely quantified to date. While anecdotal evidence suggests that these complications and costs are much higher than in normal pregnancies no peer reviewed data are available for documentation. This is a critical question to explore since such complications have not only financial and social costs, but may raise ethical issues for prospective parents, physicians, and hospitals. These issues need to be quantified and clarified, so that proper information and counseling/guidance can be provided to the potential parents and to women wishing to be surrogates.

In 2012, the Society for Assisted Reproductive Technology reported that among 379 of their member clinics, 165172 cycles or procedures involving *in vitro* fertilization were performed, and that infants conceived using *in vitro* fertilization procedures constituted 1.5% of all births in the United States[6]. However, the number of infants being born using either traditional or gestational surrogacy is not known. For 2009, the Centers for Disease Control and Prevention (CDC) released information regarding 145244 assisted reproductive procedures performed

in the United States. California ranked the highest with 18405 procedures performed, with 7545 infants born from the use of these technologies. Only 52.7% of the infants born were singletons - in contrast to 96.8% of naturally conceived infants[7], and these data did not distinguish between surrogate and other IVF births.

IVF pregnancies are considered high-risk pregnancies due to the increased risk of prematurity, pregnancy related complications, and increased incidence of multiple gestations. These factors may directly relate to the increased medical charges associated with these pregnancies[8]. There are multiple costs specific to surrogacy, many of which are beyond the purview of this report, which focuses on the hospital costs associated with surrogate births. For example, the costs of acquisition of surrogate or gestational carrier women (often through the use of agencies who advertise for eligible women), attorneys who specialize in preparing contracts between prospective parents and the surrogate, and other costs such as specialized social services, psychological counseling for the intended parents and often for the surrogate herself.

We hypothesized that hospital charges for maternity and newborn care would be significantly greater for women serving as surrogates than those delivering after natural conception and that the hospital charges for the infants would also be significantly greater than for infants delivered after natural conception and at term among naturally conceived infants. As a major medical center in Southern California we believe that baseline data from our center may be useful in informing those contemplating surrogacy pregnancies.

MATERIALS AND METHODS

The Institutional Review Board of Loma Linda University evaluated this study and determined that it was exempt from informed consent. Selected maternity diagnosis, mode of delivery, duration of hospitalization, and hospital charges were collected from women who were identified by their obstetrical provider as being a surrogate (traditional or gestational carrier). Infants born of these pregnancies had their birth weights, gestational age, length of hospital stay, and hospital charges tabulated, as well as their stay in either the normal nursery or neonatal intensive care unit between January 1, 2012 and December 31, 2013 tabulated from medical chart review. All hospital charges data were independently tabulated by the Office of Finance based on the surrogate's or infant's medical record number, as well as, the source of payment such as private payment, third party insurer, or charged to a national health insurance scheme for international surrogacy arrangements.

Charges associated with the IVF cycles, artificial insemination, or embryo(s) transfer into the surrogate were not considered in the maternity charges. A ratio contrasting the maternity hospital charges for the surrogate carrier was compared as a ratio to the mean charges for 2540 infants delivered in 2013 after natural conception. 2013 was chosen as the baseline hospital charges for both maternity and newborn care, as the electronic medical system and financial accounting system change occurred in late December 2012. Between 2012 and 2013 there was a 9% increase in hospital charges. Therefore hospital charges for both maternity care for 2012 were adjusted by this increase in hospital charges. Charges for infant care in "normal nursery" or in the Neonatal Intensive Care unit were similarly tabulated and charges for 2012 adjusted to charges in 2013 because of the increase in hospital charges.

Table 1 Characteristics of Surrogate Women prior to surrogate pregnancy: mean, range and SD

Surrogates	Age (yr)	Gravidity	Parity
n = 45	27	2.7	2.3
Range	20-43	1-8	1-7
SD	4.6	3.6	3.3

RESULTS

According to the CDC, in 2011 and 2012 there were 1766 cycles in gestational carriers in the State of California that resulted in the birth of 1067 infants of whom 36% (in 2011) and 39% (in 2012) were born prematurely. Approximately 15% were multiple births (CDC)[9]. Data from traditional surrogacy pregnancies or outcomes are not collected by either the CDC or by the California Department of Health Services.

At our center, 45 women served as surrogates (24 gestational and 21 traditional) from January 1, 2012 until December 31, 2013. These women averaged 27 (range 20-43) years of age with a mean of 2.7 prior pregnancies prior to being a surrogate during the 24 months of our study (range 0-8 previous pregnancies). These women had an average of 2.3 living children (range 1-7) prior to the surrogate pregnancy. These data (and standard deviations) are summarized in Table 1.

According to maternity documents, prenatal care began in the 4.5 wk of embryo transfer or artificial insemination. Among women delivering at our center with embryo transfers (genetically related or not) 55.5% were with multiple embryos. Sperm from the intended father[7], donor semen[3], or mixed sperm from one male couple were impregnated into the 21 traditional surrogates. The cesarean section rate was 52% for surrogate gestations contrasted to 33% among women who conceived naturally. This increased operative mode of delivery may account for the increased average length of hospitalization among women who were surrogates. Table 2 documents the births as singleton or plural births, surrogate length of stay (LOS) for maternity care pre and post birth, and hospital charges as a ratio to women who delivered after natural conception. In the only triplet gestation

Table 2 Maternal characteristics for surrogate pregnancies related to singleton, twin or triplet delivery

Surrogates	Maternity LOS (d)	Ratio	Hospital charges (± SD)	Ratio
Singleton births (n = 20)	4.2 (1.2)	1.3	$31115	1.2
Twin births (22)	3.5 (0.8)	1.1	$29692 ± 11892	1.1
Triplet births	15	4.7	$102673	3.8

Hospital Length of Maternity Stay (LOS) and charges compare surrogate carrier charges related to LOS and maternity charges for naturally conceived term infants requiring normal nursery care (mean ± SD).

there was a significantly longer length of stay and her maternity charges were considerably higher than compared to either singleton, or twin gestations.

Sixty-nine live-born infants resulted from surrogate gestations. Four infants died soon after birth due to extreme prematurity (although the legalized parents refused resuscitation for 24 wk twins). There was one fetal death in a twin pair, and the surviving infant was classified as a singleton, and among a triplet gestation there was fetal reduction of one fetus, and the infants born were classified as twin. Among the 69 infants born, 78% were born prior to 37 completed wk and 17.4% were born less than 30 wk. The mortality rate was 5.7% among infant born using assisted reproduction technologies in contrast to 0.7% of naturally conceived infants and having their initial admission to the normal nursery. Table 3 documents the infant characteristics by birth weight, gestational age, length of hospitalization, and the ratio of charges compared to naturally conceived infants. Compared to naturally conceived singleton or twin infants admitted to the normal nursery with a mean length of stay of 2.1 d, infants delivered of surrogates had a substantially greater length of stay. This longer length of stay was undoubtedly associated with the greater number of infants admitted to the NICU after delivery to a surrogate. Hospital charges were increased 26 times for both singleton and twin deliveries (tabulated per infant) to surrogates, and 173 times for each triplet infant (the sole triplet set that were born alive).

DISCUSSION

Data regarding outcomes of surrogacy pregnancies in California using a gestational carrier and from our center (both gestational and traditional surrogates) reveal a higher rate of prematurity and lower birth weight than among pregnancies resulting from natural conception. The higher cesarean rate may be explained by the higher multiple gestation pregnancies among surrogates and is consistent with the report on the increasing cesarean section rate among twins[10].

Charges for hospital services for these women and the infants delivered provide new information regarding the consumption of medical services by these pregnancies. A discussion of healthcare economics is relevant to the data presented by our experience at a single center. While many healthcare economic discussions center on dwindling reimbursement, the issue is quite different with provision for services to surrogates. Commercial insurance coverage was available for all but one of the women serving as surrogates, and of the 69 infants all but 8 also had commercial insurance with the other women or infants classified as "self pay" resulting in a net profit for our center for maternity care. Newborns were similarly covered except that national health plans in France and Spain would not cover the costs of neonatal intensive care. Combining a well-insured population with a profitable service line such as neonatal intensive care at our center produces a favorable financial outcome for our center. However, in an environment where state-sponsored insurance payments are declining and more people are migrating towards lower-paying insurance exchanges, medical centers are inclined to protect their major sources of margin. This raises the concern of the "moral hazard" of surrogacy. As illustrated surrogate women and the infants delivered have greater rates of cesarean section, premature birth, and low birth weight infants at significantly higher rates than the population of infants born after natural conception. The same is true for IVF/Assisted Insemination pregnancies[8]. Kissin *et al*[11] recently calculated the increased medical costs attributed to Assisted Reproductive Technologies by state. California led with this economic burden for 2013 estimated at $158800418.

A "moral hazard" occurs when the system that helps create the higher risk pregnancy also stands to profit from the additional care that the women and babies are likely to require. The interests of the 3 decision-making parties - intended parents, healthcare system and insurance system - are not aligned. Although gestational surrogacy represents a fraction of all IVF related births, these increased costs and potential profitability are not aligned with value-based health care. The overwhelming desire of prospective parents is to have a normal infant ideally delivered at term. In most cases, these couples, or even single adults will have attempted multiple other means of having a child before settling on the significantly more complicated method of hiring a surrogate. Most families will be paying cash for the surrogate pregnancy ($20-30000 for a surrogate, if an egg donor is required another $5-10000, the fertility clinic and reproductive endocrinologist $15000 per cycle, the surrogacy agency $10-20000) and attorneys fees of about $10000[12]. However, the cost for prenatal care, maternity charges, and expenses associated with neonatal intensive care may exhaust some intended parents resources. While many intended parents may be able to afford the $50000 or so to begin a pregnancy with the assistance of a surrogate, we have encountered many who have been unprepared for the charges associated with the care of

Table 3 Infant characteristics after birth from surrogate pregnancy

Infant(s)	Birth weight	GA	LOS	Hospital charges	Ratio
Singleton (n = 19)	3798.3 ± 832.9	35.9 ± 2.9	11 ± 3	$154874 ± 326415	26.2
Twins (n = 44)	2151.5 ± 750.5	33.8 ± 4.3	12.7 ± 4	$154885 ± 339442	26.2
Triplets (n = 3)	1337.2 ± 91.8	30.0 ± 0	75.0 ± 0	$1025927 ± 99097	173.8

Hospital charges are expressed as a ratio of hospital charges for per infant compared to hospital charges for a term infant provided care in the normal nursery (mean ± SD). GA: Gestational age; LOS: Length of stay.

a complicated newborn born prematurely and requiring several days in a Neonatal Intensive Care unit. Nor are families necessarily prepared for all the implications of a multiple-birth and the associated short- and long-term costs. If a pregnancy has a lower than normal probability of success or more potential complications how extensive should physicians explain these risks? How much do intended parents need or want to know regarding potential complications in the newborns and the added financial costs associated with a premature infant or multiple births? These questions are central to the ethical debate that has surrounded surrogacy. Kissin *et al*[13] has stressed that outcomes of assisted reproductive technologies should properly be assessed on the basis of the number of singleton infants born at term not simply based on live births.

An extension of the "moral hazard" concerns with surrogacy has been the misunderstandings that arise between intended parents and surrogates, and unforeseen events during such a pregnancy. Intended parents-surrogates disputes have arisen when the intended parents demand that the surrogate terminate a pregnancy when a significant fetal malformation is identified, or intended parents change their mind mid-gestation, *e.g.,* by initiating divorce proceedings, or when an intended parent dies. Surrogates may make greater demands on intended parents when multi-fetal gestations occur, or they may wish to engage in behaviors forbidden in their contract, or they may wish to parent the infant themselves. As noted by Andrew W. Vorzimer, a prominent attorney in arranging such contracts in Los Angeles, of 118 surrogacy cases in which a dispute arose 82 were cases in which the intended parents changes their mind and the remainder were by women serving as surrogates (many of whom were traditional surrogates providing her eggs and also carrying the infant) (Andrew W. Yorzimer, J.D., personal communication July 18, 2013).

Margalit[14], an attorney, argues that surrogacy contracts are both desirable and necessary to ensure fairness and enforceability to the benefit of all parties involved. To increase the likelihood that these dual goals of fairness and enforceability are achieved, Margalit[14] further argues that all parties should have independent legal representation from the start of the process as well as thorough, precise, medical guidance as to the risks and probabilities of various outcomes, including catastrophic outcomes. In addition, the paper argues that both sides should receive social and psychological support, and the contract should comprehensively deal with all possible outcomes, including unhealthy newborn(s), premature birth, complications/chronic diseases, and the divorce/death of the intending parents. Finally, every effort should be made to ensure that the disparity in economic strength between the parties to the contract does not interfere with the parties decision to enter into the contract nor "interferes with their free will". Additional legal/ethical risk may arise when prospective parents turn to off-shore surrogacy agencies (primarily in India, Thailand and Mexico) in an effort to cut costs. While these agencies often charge approximately half of what United States agencies do, some are not as reputable and engage in unethical practices and sometimes out-right fraud[15].

Finally, what is the insurance company's piece of this puzzle? By and large, families have borne the expense of the surrogacy, but the infant is now covered under the family's insurance plan even though the parents have voluntarily assumed more than the usual risk. The health insurance industry has thus far been slow to adjust premiums to risk profiles. However, as responsibility for payment continues to shift over to patients through high-deductible plans and cost-sharing, it's reasonable to expect that voluntary assumptions of greater risk will be looked at more critically by the insurance industry and by state health exchanges that must assume even greater risk.

A potential game-changer to the surrogacy moral hazard is an ongoing shift in how hospitals contract with insurers. Historically, they have been paid on a fee-for-service basis where they are paid a percentage of charges or a per diem rate. As their usage increases so does their payment. Medicare saw tremendous opportunity for abuse under their cost-plus reimbursement in the 70s and switched to a DRG-based case rate that also affects Medicaid (MediCal) hospital payment in California. Recently a number of state Medicaid programs followed suit with All Patient Refined DRG-based case rate payments. However, by and large, providers are still financially incentivized to increase rather than decrease the cost of care.

Increasingly health insurance policies are requiring consumers to be more accountable for their healthcare or they are charged larger premiums.

Another aspect of the "moral hazard" of surrogacy is that voluntary risk acceptance could come increasingly under extreme scrutiny. If a medical center stood not to gain, and rather potentially to lose a great

deal in the care of surrogate women and the infants from these pregnancies (as may occur in some cases of international prospective patents counting on reimbursement from their countries national health plan, especially countries that deem surrogacy illegal) how might this impact the market for the care of women surrogates, or their infants? All of these dynamic considerations make it imperative that prospective parents and medical providers have a full understanding of the risks and frequently unforeseen costs associated with surrogacy decisions.

In conclusion, data from California indicate that gestational surrogacy is increasing, and data highlight the substantial increase in multiple births, often born prematurely in California. We document at our single site the extensive requirement for neonatal intensive care and associated increased hospital charges for medical services for both surrogate (both gestational and traditional) and infants from surrogate pregnancies. In a value-based health care system, the "moral hazard" associated with promotion of surrogacy and the higher charges associated with maternity and infant care raises important issues in an area of health care services lacking regulation.

COMMENTS

Background

Surrogate pregnancies result in increased maternity costs in spite of pre-selected for maternal reproductive health primarily associated with an increase in multiple gestations that are associated with increased cesarean section rates, more preterm deliveries, increased neonatal intensive care with added neonatal morbidities.

Research frontiers

Surrogate pregnancies are permitted in several United States states, but the outcomes of these pregnancies have not been rigorously evaluated in terms of maternity or neonatal complications or hospital associated charges.

Innovations and breakthroughs

California has more surrogate pregnancies of any United States states and the impact on health economics is imperative for healthcare value with significantly greater multiples births than occur have natural conception.

Applications

Health economists and insurance providers are focused on health care value. Given the increased charges associated with surrogate pregnancies and the infants born thereof, surrogacy may come under additional scrutiny because of the moral hazard created by these gestations and the impact on health care resources.

Terminology

In this paper surrogacy includes both traditional and gestational surrogacy.

Peer-review

The authors have performed a good study, the manuscript is interesting.

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Ovarian simple cysts in asymptomatic postmenopausal women detected at transvaginal ultrasound: A review of literature

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Abstract

AIM: To answer some questions related to the problem of ovarian simple cysts in asymptomatic postmenopausal women.

METHODS: A literature search and systematic review using MEDLINE (PubMed) database from 1980 to 2014 was performed using the following terms: "simple cyst", "postmenopause", "postmenopausal", "ultrasound", "ovary", "ovarian", "asymptomatic". Papers not related to the topic, reviews, letters to editor, opinion letter, commentaries and studies published in non-English language were excluded. Two authors then reviewed the full paper of all the studies initially selected. This review does not claim to be a meta-analysis. Therefore, meta-analysis statistics were not applied and PRISMA guidelines were not strictly followed. Simple descriptive statistics were used providing absolute numbers and corresponding percentages as well as range.

RESULTS: Nine papers were ultimately included in this review, accounting for 98899 postmenopausal women. We have found that ovarian simple cysts are relatively common in asymptomatic postmenopausal women (prevalence: 8.7%). The risk of malignancy is very low (0.19%). More than 90% of these cysts were smaller than 5 cm. Bilaterality rate ranged from 3.7% to 15%. Histologically, most cysts are serous cystadenomas (61%). When managed conservatively, a significant number resolve spontaneously (46.1%) or remain unchanged (39%).

CONCLUSION: According to these data, conservative management should be the first option to offer to these women.

Key words: Ovary; Simple cyst; Diagnosis; Cancer; Management

Core tip: The problem of ovarian simple cysts in asymptomatic postmenopausal women remains a controversial issue in gynecological practice. We have performed a literature review about this topic. We have found that ovarian simple cysts are relatively common in asymptomatic postmenopausal women. The risk of malignancy is very low. Histologically, most cysts are serous cystadenomas. When managed conservatively, a significant number resolve spontaneously or remain unchanged.

Alcazar JL, Martinez N, Juez L, Caparros M, Salas A, Errasti T. Ovarian simple cysts in asymptomatic postmenopausal women detected at transvaginal ultrasound: A review of literature. *World J Obstet Gynecol* 2015; 4(4): 108-112 Available from: URL: http://www.wjgnet.com/2218-6220/full/v4/i4/108.htm DOI: http://dx.doi.org/10.5317/wjog.v4.i4.108

INTRODUCTION

The management of adnexal simple cysts in asymptomatic postmenopausal women remains a controversial issue in gynecological practice. Albeit management of these lesions has certainly evolved from a systematic surgical removal to a more conservative approach[1], many gynecologist are still reluctant to advise such a conservative management.

Some issues regarding to this entity remains unresolved such as the lesion's size above which surgery should be advised, the risk of torsion, what factors are associated with the appearance of this lesion and for how long follow-up should be performed in case of conservative management and which control periodicity should be advised, what factors are associated to the appearance of this lesion. Most importantly, if the lesion is left *in situ,* which is the actual risk for developing a malignancy?

For this reason we decided to conduct a systematic review on the literature in an attempt to answer the following questions: (1) What should be considered an ovarian simple cyst? (2) What is the prevalence of ovarian simple cysts in asymptomatic postmenopausal women? (3) Which are the characteristics of these lesions? (4) What are the factors associated to the appearance of these lesions? (5) What is the natural history of these lesions if conservative management is chosen? (6) What is the most frequent histology if surgery is performed? (7) What is the risk of developing a malignancy? (8) What are the features associated with malignancy? and (9) What is the role of CA-125?

MATERIALS AND METHODS

A systematic review of the literature in MEDLINE (PubMed) database was performed by one of the authors (JLA) from 1980 to 2014 was performed using the following terms: "simple cyst", "postmenopause", "postmenopausal", "ultrasound", "ovary", "ovarian", "asymptomatic". This author screened titles and abstracts and excluded those papers not related to the topic, reviews, letters to editor, opinion letter, commentaries and studies published in non-English language.

Two authors (JLA, NM) then reviewed the full paper of all the studies initially selected according to the following criteria:

Inclusion criteria: (1) Study design: prospective cohort study; (2) Patients: postmenopausal asymptomatic women.

Exclusion criteria: (1) Sample size < 50 women; (2) Incomplete data about patients characteristics, follow-up, histology and malignancy rate; and (3) Ultrasound performed by transabdominal route. We decided exclude these studies because of the possibility of missing papillary projections using this route is higher and some not truly simple cysts could be as such. Disagreement between reviewers was solved by consensus.

This review does not claim to be a meta-analysis. Therefore, meta-analysis statistics were not applied and PRISMA guidelines[2] were not strictly followed. Simple descriptive statistics were used providing absolute numbers and corresponding percentages as well as range.

Additionally, since this is a review Institutional Review Board was waived.

RESULTS

A total of 79 papers were found after database search. First screen excluded 62 papers for the following reasons: paper not related to the topic (n = 42), review paper (n = 12), letter to editor or commentary paper (n = 4) and non-English language (n = 4).

Full paper was reviewed for 17 studies. Eight papers were excluded for the following reasons: retrospective design (n = 3), ultrasound evaluation performed by transabdominal route (n = 2), incomplete data (n = 3).

Therefore, nine papers were ultimately included in this review[3-11].

What should be considered as an ovarian simple cyst?

The vast majority of studies selected defined as simple cyst the presence of an oval or rounded anechoic thin-walled cyst without any irregularity in the internal wall (Figure 1)[1]. The presence of any wall irregularity or echogenic cyst's content should not be considered as a simple cyst (Figure 2).

What is the prevalence of ovarian simple cysts in asymptomatic postmenopausal women?

The nine studies included accounted for 98899

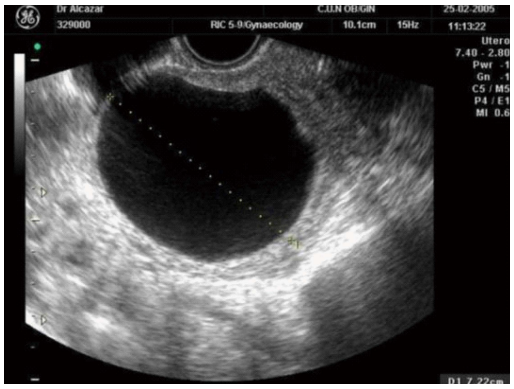

Figure 1 Transvaginal ultrasound showing an ovarian simple cyst as a round anechoic thin-walled cyst with no irregularity on the internal wall.

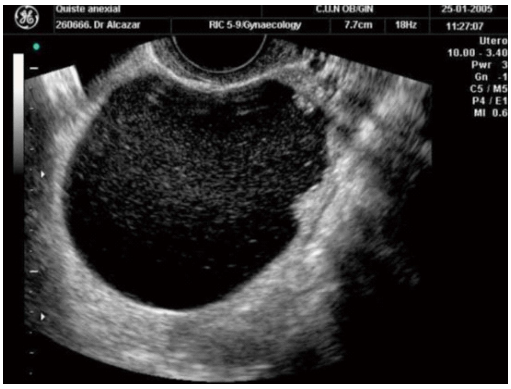

Figure 2 Transvaginal ultrasound showing a lesion that should not be considered as simple cyst, since papillary projection can be seen arising from the wall.

postmenopausal women. Two studies included only women with simple cysts[3,7]. Mean age reported in six studies, ranged from 55.5 years old to 65 years old.

The total number of simple cysts reported was 8600. Therefore, overall prevalence was 8.7% (95%CI: 8.5% to 8.9%). However, prevalence varied among different studies, ranging from 2.5% to 14.1%. Two studies with long term follow-up reported that the annual incidence of simple cysts ranged from 3% to 8%[10,11].

Which are the characteristics of ovarian simple cysts?

Most studies included cysts smaller than 5 cm in size[3-5,10,11]. However, three studies included cysts up to 10 cm[6,8,9] and one even up to 20 cm[7]. According to data derived from these studies more than 90% of these cysts were smaller than 5 cm.

Bilaterality rate was reported in three studies[8-10], ranging from 3.7% to 15%. Moreover, three or more cysts in the same woman has been reported in 5% of the cases[10].

What are the factors associated with the appearance of simple cysts?

Three studies have evaluated factors that could be associated to with the appearance of ovarian simple cysts in postmenopause.

Modesitt *et al*[8] reported that these lesions appeared more frequently in women under hormone replacement therapy (either estrogens alone or estrogens plus progestins) and in women with recent menopause.

Greenlee *et al*[10] performed a more exhaustive analysis in a large population. They found that ovarian simple cysts were associated with age (the younger the woman the higher the risk for simple cyst), non-smoking, previous history of surgery for benign gynecologic condition, previous history of ovarian benign cyst, higher education, precocious menopause and first pregnancy before 20 years old. No association was found with use of hormone replacement therapy, use of hormonal contraception, parity or history of infertility.

Sharma *et al*[11] found that previous hysterectomy, age and weight were significantly associated with simple cysts.

What is the natural history of these lesions if conservatively managed?

Data about the natural history of ovarian simple cysts in asymptomatic postmenopausal women on follow-up has been reported in eight studies[3-6,8-11]. The mean follow-up ranged from 18 to 71 mo.

Ovarian simple cysts resolved spontaneously in almost half of the cases (46.1%). Spontaneous resolution rate ranged from 0% to 69.4%, depending on the study.

Factors associated to resolution were age (the younger the woman the higher the probability of resolution)[6,9], cyst's size (the smaller the size the higher the probability of resolution)[5], the number of cysts (more than 2 cysts was associated to a lower probability of resolution)[10].

Among those cysts that have not resolved, many number did not change in size or aspect (39%; range 23% to 96%[3,4,5,8,9,11]). Among those that changed in appearance most of them developed septations or solid areas[5,8].

What is the most frequent histology in those surgically removed?

Detailed data about histology of surgically removed cysts were reported on six studies[3,5-9]. The total number of cysts removed was 525. Three hundreds and thirty-six were removed after diagnosis and 189 during follow-up. Only on study reported median time elapsed from diagnosis up to removal (27 mo)[9].

Simple cyst and serous cystadenoma were the most frequent histologies (61%, 155/254) followed by cystadenofibroma (8%, 20/254) and para-ovarian cyst (5%, 14/254).

Other reported histologic diagnoses were endometrioma, dermoid cyst, hydrosalpinx and mucinous

cystadenoma.

What is the risk of developing a malignancy?

Overall 16 malignancies among 8600 simple cysts were reported[7,9-11]. This represents a malignancy rate of 0.19%.

Greenlee *et al*[10] reported on 15735 women enrolled in the PLCO (Prostate, Lung, Colorectal and Ovarian Cancer) Screening Trial. Nine women (0.41%) among those 2217 diagnosed as having a simple cyst developed an ovarian cancer. Whereas, 55 out of 12638 (0.44%) without a simple cyst developed an ovarian malignancy. The relative risk for those with ovarian simple cyst was 0.93 (95%CI: 0.46-1.88).

Sharma *et al*[11] reported on 48230 women enrolled in the UKCTOCS (United Kingdom Collaborative Trial of Ovarian Cancer Screening) study. At first scan, 22194 women had normal ovaries visualized, 21943 women had non-visualized one or both ovaries, 1234 women had an inclusion cyst (simple cyst < 10 mm) and 197 had an inclusion cyst and a simple cyst (simple cyst > 10 mm). After a median follow-up of 3 years, and considering only women with both ovaries visualized and those with inclusion and/or simple cyst, three women (0.2%) with only inclusion cyst and one woman (0.5%) with inclusion and simple cyst developed an ovarian malignancy. Twenty nine out of 22194 women in whom both ovaries were visualized and were normal developed an ovarian cancer. The relative risk for women with inclusion cyst was 1.92 (95%CI: 0.62-5.92) and relative risk for women with inclusion cyst and simple cyst was 4.01 (95%CI: 0.69-22.92).

What are the features associated to malignancy?

This is probably the most interesting question. However, the information provided in the studies evaluated for this mini-review is scanty. From data reported from three studies[7,9,11] including eight ovarian cancers, we observed that most of them (6/8) were stage Ⅰ and in 5/8 the size of the cyst was < 5 cm in 5 out of 8 cases.

What is the role of CA-125?

Tumor marker CA-125 was used in all studies but two[7,11]. Some studies established normal CA-125 level (< 35 UI/mL) as an inclusion criteria[4,8-10] and most of them assessed CA-125 during follow-up[3-6,9,10]. However, three studies did not report specific information about CA-125 levels[6,8,10].

Those studies that reported data about CA-125 during follow-up reported no significant changes of this tumor marker during follow-up, even in those cases with sonographic changes in the ovarian simple cyst[3-5]. In the study by Castillo *et al*[9], there was one case of ovarian malignancy. In this case CA-125 raised during follow-up (45 UI/mL).

DISCUSSION

In this systematic review, we have addressed the issue of ovarian simple cysts in asymptomatic postmenopausal women.

We have found that this type of lesions is common in this group of women. This finding is in agreement with data from autopsy studies[12,13]. Another finding in agreement with autopsy studies is that most cysts are small and that the most common histology is simple cyst or serous cystadenoma.

Transvaginal ultrasound route is essential for ascertaining that the lesion is actually a simple cyst, since small papillary projections might be missed using transabdominal ultrasound[14].

Patient's characteristics consistently associated with the presence of a simple ovarian cyst in postmenopausal women are age and previous history of pelvic or gynecologic surgery. This, in fact, is not surprising since younger postmenopausal women may have residual ovarian activity and many of these simple cysts could be "functional cysts" developed from this residual activity. This fact may also explain why the probability of spontaneous resolution is higher in women with recent menopause. On the other hand, previous pelvic surgery increases the risk of peritoneal cysts that may resemble ovarian simple cysts.

No study reported data about complications such as torsion or rupture. Therefore, albeit it is not possible to draw definitive conclusions regarding these complications. However, it seems that they are uncommon in ovarian simple cysts during the menopause.

Additionally no study reported information about when and for how long follow-up should be performed in those cysts that had not resolved spontaneously.

Regarding surgery in most studies there is no data reported about the surgical approach. The authors think that laparoscopic cyst removal should be the best approach when surgery is decided[15].

Probably one of the most important findings in our review is that related to the risk of malignancy of these lesions. Actually, in the two largest studies published so far the relative risk of developing an ovarian malignancy in postmenopausal women with ovarian simple cyst was not increased as compared with those postmenopausal women without ovarian simple cyst.

COMMENTS

Background

Postmenopausal simple cysts in asymptomatic women management remains as an unsolved issue in gynecological practice. This is relevant because of surgery has been traditionally performed. However, recent data challenge this concept showing that conservative management should be offered to these women.

Research frontiers

A number of papers have been published addressing this question in the last fifteen years. However, adequate systematic reviews are lacking.

Innovations and breakthroughs

The paper offers an updated systematic review about this topic. According to the authors' findings in this mini-review, it could be assumed that ovarian simple cysts are common in asymptomatic postmenopausal women. The risk of malignancy is very low and having a simple ovarian cyst does not seem to increase the risk of developing an ovarian cancer. Many of them will resolve spontaneously or will remain unchanged during follow-up. The role of CA-125 at diagnosis and for following-up remains to be determined. Data about the use of CA-125 are scanty in the studies reviewed. Most studies used CA-125 as inclusion criteria and during follow-up. In most cases CA-125 did not change, even in those cysts that changed during follow-up. Only in one case of carcinoma the value of CA-125 was reported as elevated. In the authors' opinion, the role of routine CA-125 assessment in the follow-up of postmenopausal ovarian simple cyst would be arguable. The authors' review has some limitations. First, the authors did not perform a formal meta-analysis. Therefore, conclusions based on statistical inferences cannot be drawn. Second, the number of studies included is small. Third, studies are considerably heterogeneous in design and data reported. However, some questions remain unanswered and future research is needed to try to answer these questions: (1) Which cysts are at higher risk for developing a malignancy? (2) Is follow-up needed for these lesions? (3) For how long these cysts should be followed-up? and (4) Which follow-up protocol should be implemented?

Applications

The results of the analysis may have a relevant clinical impact since the authors have shown that ovarian simple cysts are common in asymptomatic postmenopausal women and the risk of malignancy is very low. These findings should modify the traditional surgical management of these lesions for a more conservative one. However, there is still a room for research since some questions remain unanswered (see above).

Peer-review

This is an interesting manuscript.

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META-ANALYSIS

Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis

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Data sharing statement: Review manager file with statistical code available from the corresponding author at lcraciunas@doctors.org.uk.

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Abstract

AIM: To appraise critically the published randomised controlled trials (RCTs) reporting on the effectiveness of using hyaluronic acid (HA) for sperm immobilisation and selection before intracytoplasmic sperm injection (ICSI).

METHODS: Two authors used the PICO Method in order to perform a comprehensive literature search of the standard medical databases in June 2015. Data from the included studies was extracted independently by two authors using a predefined pro-forma. Review Manager (RevMan) was used to calculate the combined outcomes where multiple studies contributed with their results. Risk ratio (RR) with a 95%CI using the Mantel-Haenszel method was calculated for binary data variables. Heterogeneity was measured using the χ^2 test and quantified using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) the combined outcome was calculated using the random effects model. The results from the meta-analysis were displayed as forest plots. The guideline of the Cochrane Collaboration was used to assess the risk of bias and it was illustrated as a risk of bias graph.

RESULTS: The systematic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. Eleven RCTs involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or polyvinylpyrrolidone (PVP)

were included in this systematic review and meta-analysis. There was low heterogeneity among the included trials (χ^2 = 16.86, df = 11, P = 0.11; I^2 = 35%). There was no statistical difference between HA and PVP groups in terms of fertilisation rate (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45), good embryos rate (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76), live birth rate (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36), clinical pregnancy rate (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53) and implantation rate (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16). The quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding.

CONCLUSION: This systematic review and meta-analysis provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI.

Key words: Hyaluronic acid; Sperm; Intracytoplasmic sperm injection

Core tip: Hyaluronic acid (HA) has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. We performed a systematic review and meta-analysis of eleven randomised controlled trials involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP. There was no difference between HA and PVP groups in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

Craciunas L, Tsampras N, Kollmann M, Stirbu L, Raine-Fenning NJ. Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis. *World J Obstet Gynecol* 2015; 4(4): 113-123 Available from: URL: http://www.wjgnet.com/2218-6220/full/v4/i4/113.htm DOI: http://dx.doi.org/10.5317/wjog.v4.i4.113

INTRODUCTION

The success of intracytoplasmic sperm injection (ICSI), as a bypass of the natural selection processes taking place in the female reproductive tract, would be impossible without advances in the laboratory preparation and identification of sperm for use with ICSI. Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome[1,2].

Hyaluronic acid (HA) is found naturally in the women's reproductive tract and it forms a component of the cumulus-oocyte complex. It has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP[3,4].

Furthermore, it has been shown that sperm's capacity to bind HA is a biochemical marker of maturity and function, suggesting the selection of sperm by HA binding to be an alternative to microscopic assessment of motility and morphology[3].

Several descriptive reviews of the current advanced sperm selection methods support the use of HA for sperm immobilisation and selection for ICSI, but none of them report a quantitative measure of the effect it has on the ICSI outcome[5-8].

The objective of this study is to appraise critically the published randomised controlled trials (RCTs) reporting on the use of HA for sperm immobilisation and selection before ICSI.

MATERIALS AND METHODS

Literature search

We used the PICO Method[9] to formulate a specific and answerable clinical question following which we performed a comprehensive literature search based on a predefined protocol. The medical subject headings (MeSH) "sperm injections, intracytoplasmic", "semen", "hyaluronic acid", "infertility", "fertilization" and "live birth" were combined with free terms "hyaluronan", "sperm", "ICSI", "PICSI", "SpermCatch", "SpermSlow", "polyvinylpyrrolidone", "PVP", "embryo quality", "pregnancy", "implantation", "costs", "adverse events" in order to search Medline/PubMed/PMC, Cochrane Central Register of Controlled Trials (CENTRAL), EBSCOhost, ClinicalTrials.gov and Google Scholar from inception until June 2015. The "Related citations" function and hand search of references were used for all relevant studies in order to identify additional RCTs.

Study selection

We set our inclusion criteria as RCTs evaluating sperm immobilisation and selection using HA before ICSI with no filter for date, country or hospital of origin, publication language, sample size or blinding. For studies presented in more than one publication, we only included the most extensive and recent version in order to avoid overlapping data.

Endpoints

The primary endpoints of the present meta-analysis were defined as: fertilisation rate, embryo quality and live birth rate. Secondary endpoints were: clinical pregnancy and implantation rates, adverse events and costs.

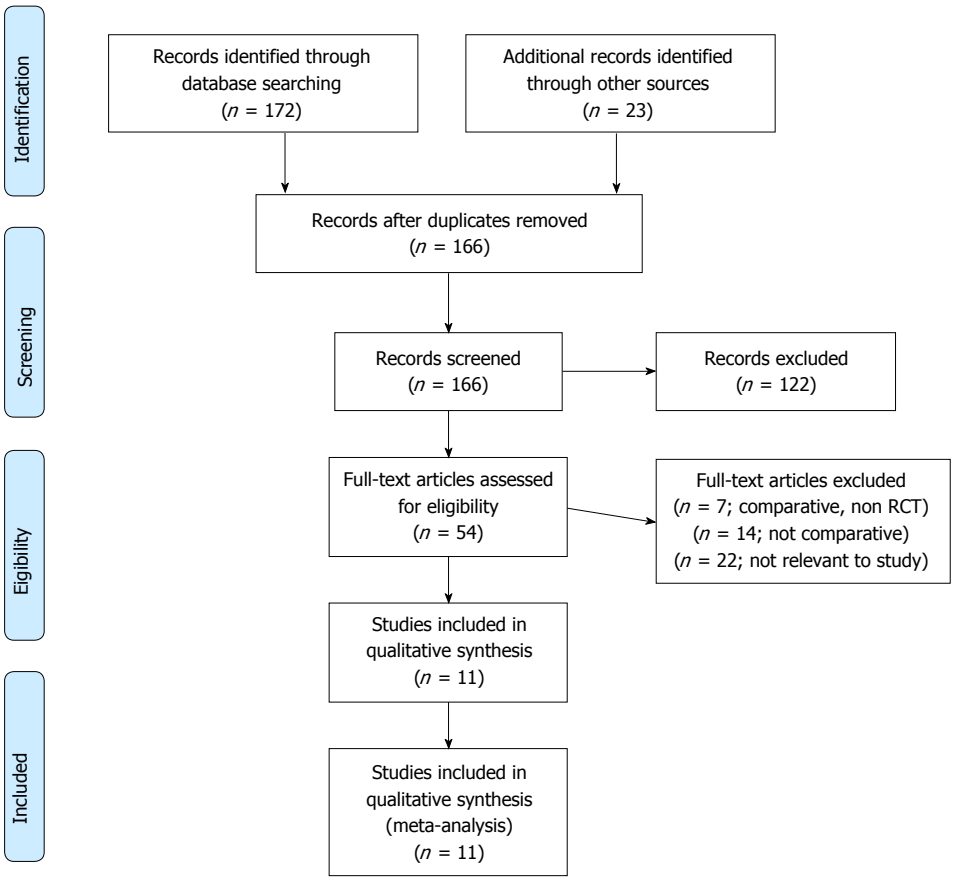


Figure 1 PRISMA flow chart showing trial selection methodology. RCT: Randomised controlled trial.

Data extraction

Two authors extracted the data following the literature search and study selection using predefined tables. Information related to first author, year of publication, country of origin, age of participants, inclusion criteria, the day of embryo transfer, number of embryos transferred, publication type, intervention protocols, number of participants, fertilisation rate, embryo quality, clinical pregnancy rate, implantation rate, live birth rate, adverse events, costs, randomisation technique, allocation concealment, blinding and data reporting was retrieved for each of the included studies. We contacted the study authors in order to obtain more data where it was required.

Statistical analysis

The software package RevMan 5.2.11[10], provided by the Cochrane Collaboration, was used for statistical analysis. We calculated the risk ratio (RR) with a 95%CI using the Mantel-Haenszel method[11] for binary data variables.

We measured the heterogeneity using the χ^2 test and quantified it[12] using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) we reported the combined outcome calculated using the random effects model[13]. Forest plots were used for the visual display of the results from the meta-analysis.

The guideline of the Cochrane Collaboration[14] was used to assess the risk of bias and it was illustrated as a risk of bias graph. GradePro (Version 3.2 for Windows) provided by the Cochrane Collaboration[15] was used to generate the summary of the evidence.

We performed subgroup analysis based on publication type (full text *vs* abstract) and type of reporting of the results (per women *vs* per cycle) for each of the variables with summated outcome.

RESULTS

The systemic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. The PRISMA flow chart to explain the RCTs selection is shown in Figure 1. The summary of the evidence is presented in Figure 2. Eleven RCTs[5,16-25] evaluating 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP were included in this systematic review and meta-analysis. There were 6926 injections in the HA group and 6793 injections in the PVP group. The characteristics of the included RCTs are shown in Table 1, and the procedure protocols used for the women in all of the RCTs are shown in Table 2. Variables used to achieve a combined outcome are shown in Table 3. One RCT[17] included four arms and we analysed the data as for two studies. Seven RCTs[5,16,18,20,22,23,25] were

Outcome	Illustrative comparative risks[1] (95%CI)		Relative effect (95%CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk Polyvinylpyrrolidone (PVP)	Corresponding risk Hyaluronic acid (HA)				
Fertilisation rate RR Follow-up: mean 8 wk	Study population 740 per 1000 Moderate 745 per 1000	747 per 1000 (733 to 762) 752 per 1000 (738 to 767)	RR 1.01 (0.99 to 1.03)	13719 (12 studies)	⊕⊕⊕⊖ moderate[1]	
Good embryos rate RR Follow-up: mean 8 wk	Study population 466 per 1000 Moderate 367 per 1000	471 per 1000 (447 to 494) 371 per 1000 (352 to 389)	RR 1.01 (0.96 to 1.06)	5834 (8 studies)	⊕⊕⊕⊖ moderate[1]	
Clinical pregnancy rate RR Follow-up: mean 8 wk	Study population 417 per 1000 Moderate 400 per 1000	433 per 1000 (383 to 487) 416 per 1000 (368 to 468)	RR 1.04 (0.92 to 1.17)	1367 (9 studies)	⊕⊕⊕⊖ moderate[1]	
Implantation rate RR Follow-up: mean 8 wk	Study population 150 per 1000 Moderate 164 per 1000	175 per 1000 (141 to 219) 192 per 1000 (154 to 239)	RR 1.17 (0.94 to 1.46)	1637 (4 studies)	⊕⊕⊕⊖ moderate[1]	
Liver birth rate RR Follow-up: mean 37 wk	Study population 253 per 1000 Moderate 263 per 1000	291 per 1000 (218 to 390) 302 per 1000 (226 to 405)	RR 1.15 (0.86 to 1.54)	473 (3 studies)	⊕⊕⊕⊖ moderate[1]	

[1]Selection, performance and detection bias due to inadequate concealment technique, blinding of personnel and outcome assessment. RR: Risk ratio.

Figure 2 Summary and strength of the evidence from trials analysed on GradePro®.

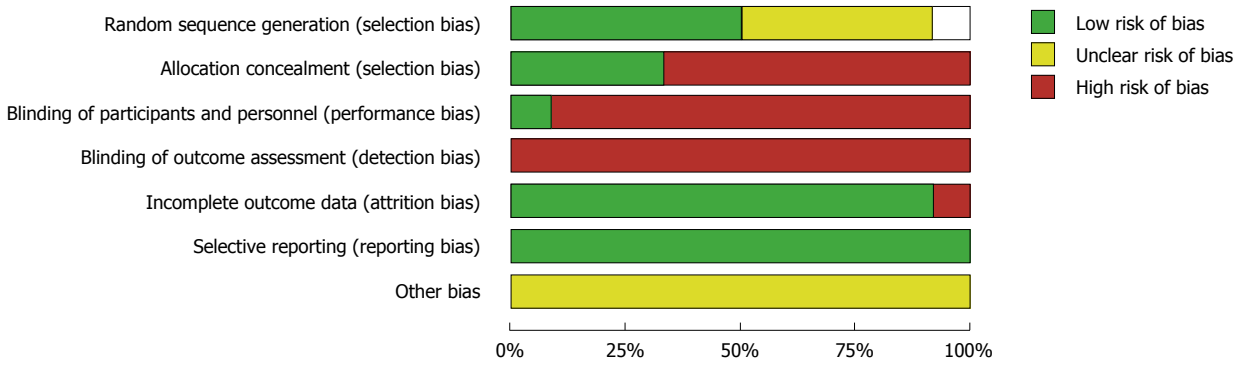


Figure 3 Risk of bias generated using cochrane risk of bias assessment tool.

published as full articles and four RCTs were published as abstracts[17,19,21,24]. Two RCTs[5,22] reported the results per ICSI cycle not per woman randomised. There was complete agreement between authors in terms of included studies and extracted data.

Methodological quality of included studies

Based upon the guidelines suggested by the Cochrane Collaboration, the quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding (Figure 3).

The combined outcome of all of the variables is given below.

Fertilisation rate per oocyte injected

All the included RCTs reported on this outcome with low heterogeneity [χ^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%] among them. The fertilisation rate was similar (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45; Figure 4A) in the HA group compared to the PVP group. The result did not change when we excluded the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.47) or when we used the random effects

Table 1 Characteristics of included trials

Trial	Country	Mean age	Inclusion criteria	Day of embryo transfer	Number of embryos transferred	Publication type
Balaban *et al*[16]						
HA	Sweden	NA	Male factor	Day 2-3	3.1	Full text
PVP					3.1	
Barak *et al*[5]						
HA	Israel	31.0	Male factor	Day 2-3	3.8	Full text
PVP		30.9			3.8	
Castillo-Baso *et al*[17]						
HA	Mexico	35.1	NA	Day 2-3 or day 5-6	NA	Abstract
PVP		35.3				
Choe *et al*[18]						
HA	Korea	35.4	Previous low fertilization rate Multiple IVF failures	Day 3 or day 5	NA	Full text
PVP						
Gandhi *et al*[19]						
HA	Spain	NA	NA	NA	NA	Abstract
PVP						
Majumdar *et al*[20]						
HA	India	31.7	Unexplained infertility, normal semen analysis	Day 2-3	2.49	Full text
PVP		31.5			2.39	
Moon *et al*[21]						
HA	Korea	NA	NA	Day 3 or day 5	NA	Abstract
PVP						
Parmegiani *et al*[22]						
HA	Italy	37.5	Sperm number > 10^6 and sperm motility > 5%	Day 2-3	2.25	Full text
PVP		37.1			2.15	
Van Den Bergh *et al*[23]						
HA	Switzerland	NA	Women younger than 38 yr with at least four metaphase Ⅱ oocytes	Day 2-3	NA	Full text
PVP						
Worrilow *et al*[24]						
HA	United States	NA	NA	NA	NA	Abstract
PVP						
Worrilow *et al*[25]						
HA	United States	33.3	Women younger than 40 years with at least four metaphase Ⅱ oocytes; Men with sperm count > 10000/mL	Day 2-3 or day 5-6	NA	Full text
PVP		33.7				

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

model for calculation (P = 0.83).

Good embryos rate per oocyte injected

Eight RCTs reported on this outcome with low heterogeneity [χ^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%] among them. There were 2714 good quality embryos obtained from 5835 oocytes injected. No statistically significant difference was found between the HA and PVP groups (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76; Figure 4B). Similar results were obtained when we excluded the RCT[22] reporting the outcomes per ICSI cycle (P = 0.56) or when we used the random effects model for calculation (P = 0.59).

Live birth rate per cycle

Three RCTs reported on this outcome with no heterogeneity [χ^2 = 0.67, df = 2, (P = 0.73); I^2 = 0%] among them. The live birth rate was similar in the HA group compared to the PVP group (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36; Figure 4C). Same results were obtained after excluding the RCT[22] reporting the outcomes per ICSI cycle (P = 0.68) or when we calculated using the random effects model (P = 0.41).

Clinical pregnancy rate per transfer

Nine RCTs reported on this outcome with no heterogeneity [χ^2 = 5.50, df = 8, (P = 0.70); I^2 = 0%] among them. The clinical pregnancy rate was similar between HA group and PVP group (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53; Figure 5A). No difference was found by excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.98) or by calculating the combined outcome using the random effects model (P = 0.68).

Implantation rate per embryo transferred

Four RCTs reported on this outcome with no heterogeneity [χ^2 = 1.12, df = 3, (P = 0.77); I^2 = 0%] among them. Similar implantation rates were obtained in the HA and PVP groups (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16; Figure 5B). Excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.67) or using the random effects model for calculation (P = 0.17) did not change the result.

Table 2 Treatment protocol adopted in included trials

Ref.	HA group	PVP group
Balaban *et al*[16]	Oocytes were injected with sperm exposed to, SpermCatch (NidaCon International, Gothenburg, Sweden), a viscous liquid containing hyaluronate and human serum albumin	Oocytes were injected with sperm exposed to the PVP-containing product
Barak *et al*[5]	Sperm suspension which contained motile spermatozoa was introduced into the center of viscous suspension of hyaluronic acid. Motility of the sperm cells was slowed down. After breaking the sperm tail with the injection pipette, the spermatozoa were injected into the oocytes	MII oocytes were injected with sperm cells which were immobilized and aspirated for injection in 10% PVP solution
Castillo-Baso *et al*[17]	Sperm selected by PICSI (Mid Atlantic Diag. Inc.)	Sperm selected by conventional ICSI
Gandhi *et al*[19]	2 μL droplet with suspension of spermatozoa was placed to a 5 μL droplet of HA-containing medium (SpermSlow; MediCult, Jyllinge, Denmark) and incubated for 15 min at 37 ℃ under oil. Spermatozoa bound to HA in the junction of the two droplets were identified and carefully detached by injecting pipette (ICSI Micropipette; TPC, Thebarton, Adelaide, South Australia) and subsequently injected into a MII oocyte	Before injection, 3 μL of sperm suspension was transferred to 7 μL of 7% polyvinylpyrrolidone (PVP; SAGE) solution to remove debris and get better control. Spermatozoa with best morphology were selected for injection into a MII oocytes using inverted microscope equipped with micromanipulators
Gandhi *et al*[19]	Donated oocytes to avoid female infertility as a bias factor, randomly carried out with SpermSlow for sperm selection	Donated oocytes to avoid female infertility as a bias factor randomly carried out with PVP for sperm selection
Majumdar *et al*[20]	Sterile PICSI dishes (Origio MidAtlantic Devices, United States) with three hyaluronan microdots attached to the interior bottom, were used. 10 μL droplets of culture medium (GMOPS, Vitrolife) were placed over the hyaluronan microdots and an elongated 10 μL drop of PVP was made below the drops, before covering the dish with oil. 1-2 μL of sperm suspension was then added to the hyaluronan microdot containing droplets. After 5 min of incubation at 37 ℃, HA bound sperm with normal morphology were removed with an injecting micropipette (TPC, Australia) to the adjacent PVP droplet, immobilized and subsequently injected	An elongated 10 μL poly vinyl pyrolidone drop (PVP, Medicult, Denmark) under oil, was used to select spermatozoa with normal morphology for subsequent injection
Moon *et al*[21]	ICSI were performed with husband spermatozoa which immobilized in 2.5 mg/mL hyaluronic acid	ICSI were performed with husband spermatozoa which immobilized in 5% PVP
Parmegiani *et al*[22]	Spermatozoa were selected for their ability to bind to HA: A 2-mL droplet with suspension of spermatozoa was connected with a pipette tip to a 5-mL droplet of HA-containing medium (SpermSlow; MediCult) and allowed to incubate for 15 min at 37 ℃ under oil (Liquid Paraffin; MediCult). Spermatozoa bound to HA in the junction zone of the two droplets were selected and easily detached by injecting pipette (ICSI Micropipette; Humagen Fertility Diagnostics) and subsequently injected into oocytes	Conventional PVP-ICSI procedure
Worrilow *et al*[24]	At the time of injection, drops were prepared in the lid of a Falcon Petri dish (353004; Becton Dickinson, Franklin Lakes, United States). In the middle of the dish a 10 μL drop of Spermslow (Medicult) and a 10 μL Flushing Medium drop (Medicult) were connected by a 3-4 mm junction bridge of medium and consecutively encircled by five 10 μL drops of Flushing medium. This setup was covered with liquid paraffin (Medicult). A 2 μL volume of prepared semen was added to the medium part of the Spermslow/Flushing medium central mixture. The spermatozoa were allowed to migrate towards the junction for a period of 15-20 min at 37 ℃. Spermatozoa were carefully selected near the junction between the sperm droplet and the SpermSlow droplet	Non-bound, forward-moving spermatozoa were taken from the SpermSlow droplet
Worrilow *et al*[24]	PICSI embryos created using Hyaluron Bond-sperm	Standard sperm selection criteria
Worrilow *et al*[25]	The final sperm suspension of HYAL patients was placed upon microdots of hyaluronan in the PICSI Sperm Selection Device (Biocoat, Inc., Horsham, PA) and overlaid with oil. Following a 5-10 min incubation period, HB sperm were selected following the manufacturer's instructions	The final sperm suspension of patients in the control group was placed into standard ICSI dishes for selection

PVP: Polyvinylpyrrolidone; ICSI: Intracytoplasmic sperm injection; HA: Hyaluronic acid.

Adverse events and costs

None of the studies reported on these outcomes.

DISCUSSION

Main findings

This systematic review and meta-analysis based on eleven moderate to low quality RCTs provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates. None of the studies reported on costs hence we could not perform a cost-effectiveness analysis.

Strengths and limitations

By performing a comprehensive literature search of the standard medical databases and grey literature

Table 3 Variables used for systematic review and meta-analysis *n* (%)

Trial	Transfers (*n*)	Fertilisation rate	Good embryos	Clinical pregnancy	Implantation rate	Live birth
Balaban *et al*[16]	Women					
HA	48	360 (72.14)	226 (50.33)	20 (41.66)	27 (18.12)	19 (39.58)
PVP	44	337 (75.05)	211 (46.99)	19 (43.18)	27 (19.14)	18 (40.90)
Barak *et al*[5]	Cycles					
HA	58	525 (72.61)	NA	29 (50.00)	41 (18.55)	NA
PVP	65	484 (74.57)		25 (38.46)	35 (14.00)	
Castillo-Baso *et al*[17]	Women					
HA	30	143 (49.14)	87 (29.89)	16 (53.33)	-34	NA
PVP	30	134 (50.95)	84 (31.93)	12 (40.00)	-24	
Castillo-Baso *et al*[17]	Women					
HA	30	140 (56.91)	105 (42.68)	14 (46.66)	-25	NA
PVP	30	163 (61.97)	99 (37.64)	13 (43.33)	-22	
Choe *et al*[18]						
HA	18 women	81 (75.70)	13 (12.14)	NA	NA	NA
PVP		93 (83.03)	15 (13.39)			
Gandhi *et al*[19]	Women					
HA	77	909 (82.33)	675 (61.14)	41 (53.24)	NA	NA
PVP	77	923 (82.48)	698 (62.37)	47 (61.03)		
Majumdar *et al*[20]	Women					
HA	71	353 (64.65)	154 (43.62)	25 (35.21)	39 (22.03)	22 (30.98)
PVP	80	371 (65.66)	170 (45.82)	28 (35.00)	36 (18.84)	21 (26.25)
Moon *et al*[21]						
HA	1 woman	18 (81.81)	11 (50.99)	NA	NA	NA
PVP		22 (78.57)	10 (35.71)			
Parmegiani *et al*[22]	Cycles					
HA	125	304 (91.56)	101 (30.42)	31 (24.80)	35 (12.41)	29 (23.20)
PVP	105	236 (85.81)	55 (20.00)	22 (20.95)	23 (10.17)	19 (18.09)
Van Den Bergh *et al*[23]						
HA	44 women	154 (75.49)	NA	NA	NA	NA
PVP		142 (69.95)				
Worrilow *et al*[24]	Women					
HA	7	77 (61.11)	NA	4 (57.14)	NA	NA
PVP	8	98 (66.66)		2 (25.00)		
Worrilow *et al*[24]	Women					
HA	237	2105 (77.21)	NA	112 (47.25)	NA	NA
PVP	245	2024 (74.41)		117 (47.75)		
Total						
HA		5169 (74.63)	1372 (46.44)	292 (42.75)	142 (17.12)	70 (28.68)
PVP		5027 (74.00)	1342 (46.59)	285 (41.66)	121 (14.97)	58 (25.32)

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

with no filters for date, country or hospital of origin, publication language, sample size or blinding we were able to identify eleven RCTs including conference abstracts in order to calculate the combined outcomes. Where the reported data was insufficient we contacted the study authors to gain extended reports.

The heterogeneity was low among the included RCTs and lead to consistent results by using both fixed effect and random effects models for calculations.

Our study is limited by the moderate to poor methodological quality of the included studies because of unclear randomisation technique, inadequate allocation concealment and blinding.

Some might argue in relation to the inclusion of the two RCTs reporting the results per ICSI cycle and not per women randomised, but we performed calculations excluding them for each of the primary and secondary outcomes, without identifying any significant difference.

Comparison with other studies and further research

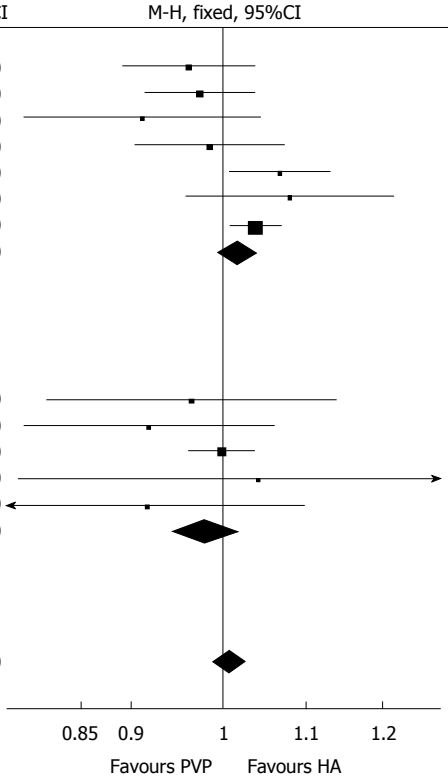
To our knowledge this is the first meta-analysis comparing HA with PVP for sperm immobilisation and selection before ICSI. By observing the forest plot for each end point one can easily notice the similarities between the RCTs as most of them cross the vertical line meaning there is no statistical difference between the groups.

Future trials should be conducted according to the CONSORT guidelines. Due to the nature of the intervention, it would be difficult to achieve blinding of the embryologist performing the sperm selection, but the risk of bias could be reduced by blinding the outcome assessors and the personnel performing the embryo transfers.

It is important to report on the possible adverse events related to less physiological immobilisation and selection of the sperm and to set live birth rate as a primary outcome for the comparisons.

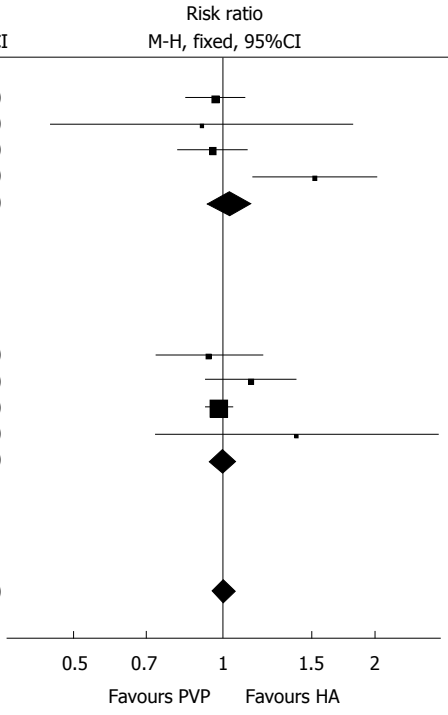
A

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.1.1 Full texts						
Balaban 2003	360	499	337	449	7.0%	0.96 (0.89, 1.04)
Barak 2001	525	732	484	649	10.1%	0.97 (0.91, 1.04)
Choe 2012	81	107	93	112	1.8%	0.91 (0.80, 1.04)
Majumdar 2013	353	546	371	565	7.2%	0.98 (0.90, 1.07)
Parmegiani 2010	304	332	236	275	5.1%	1.07 (1.01, 1.13)
Van Den Bergh 2009	154	204	142	203	2.8%	1.08 (0.96, 1.22)
Worrilow 2013	2105	2726	2024	2720	39.9%	1.04 (1.01, 1.07)
Subtotal (95%CI)		5137		4973	73.9%	1.02 (0.99, 1.04)
Total events	3882		3687			
Heterogeneity: Chi^2 = 12.23, df = 6 (P = 0.06); I^2 = 51%						
Test for overall effect: z = 1.48 (P = 0.14)						
1.1.2 Abstracts						
Castillo-Baso 2011 a	143	291	134	263	2.8%	0.96 (0.82, 1.14)
Castillo-Baso 2011 b	140	246	163	263	3.1%	0.92 (0.79, 1.06)
Gallego 2013	909	1104	923	1119	18.1%	1.00 (0.96, 1.04)
Moon 2000	18	22	22	28	0.4%	1.04 (0.79, 1.37)
Worrilow 2006	77	126	98	147	1.8%	0.92 (0.77, 1.10)
Subtotal (95%CI)		1789		1820	26.1%	0.98 (0.94, 1.02)
Total events	1287		1340			
Heterogeneity: Chi^2 = 2.40, df = 4 (P = 0.66); I^2 = 0%						
Test for overall effect: z = 1.03 (P = 0.30)						
Total (95%CI)		6926		6793	100.0%	1.01 (0.99, 1.03)
Total events	5169		5027			
Heterogeneity: Chi^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%						
Test for overall effect: z = 0.75 (P = 0.45)						
Test for subgroup differences: Chi^2 = 2.67, df = 1 (P = 0.10); I^2 = 62.6%						



B

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.2.1 Full texts						
Balaban 2003	226	499	211	449	16.5%	0.96 (0.84, 1.11)
Choe 2012	13	107	15	112	1.1%	0.91 (0.45, 1.82)
Majumdar 2013	154	353	170	371	12.3%	0.95 (0.81, 1.12)
Parmegiani 2010	101	332	55	275	4.5%	1.52 (1.14, 2.03)
Subtotal (95%CI)		1291		1207	34.3%	1.03 (0.93, 1.14)
Total events	494		451			
Heterogeneity: Chi^2 = 9.02, df = 3 (P = 0.03); I^2 = 67%						
Test for overall effect: z = 0.59 (P = 0.55)						
1.2.2 Abstracts						
Castillo-Baso 2011 a	87	291	84	263	6.5%	0.94 (0.73, 1.20)
Castillo-Baso 2011 b	105	246	99	263	7.1%	1.13 (0.92, 1.40)
Gallego 2013	675	1104	698	1119	51.4%	0.98 (0.92, 1.05)
Moon 2000	11	22	10	28	0.7%	1.40 (0.73, 2.68)
Subtotal (95%CI)		1663		1673	65.7%	1.00 (0.94, 1.06)
Total events	878		891			
Heterogeneity: Chi^2 = 2.96, df = 3 (P = 0.40); I^2 = 0%						
Test for overall effect: z = 0.11 (P = 0.91)						
Total (95%CI)		2954		2880	100.0%	1.01 (0.96, 1.06)
Total events	1372		1342			
Heterogeneity: Chi^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%						
Test for overall effect: z = 0.30 (P = 0.76)						
Test for subgroup differences: Chi^2 = 0.31, df = 1 (P = 0.57); I^2 = 0%						



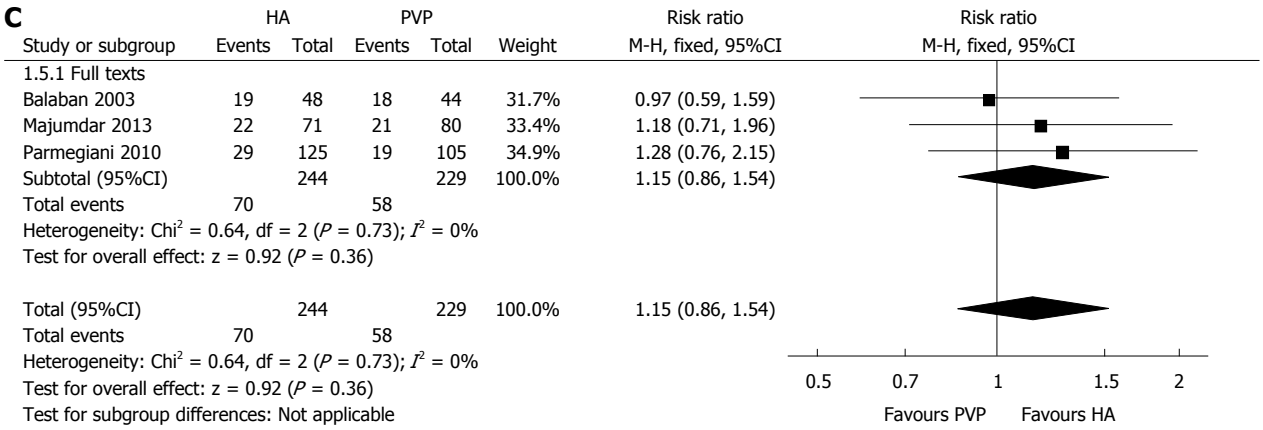


Figure 4 Primary endpoints. A: The forest plot showing fertilisation rate per oocyte injected. Risk ratio is shown with 95%CI; B: The forest plot showing good embryos rate per oocyte injected, risk ratio is shown with 95%CI; C: The forest plot showing live birth rate per cycle. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

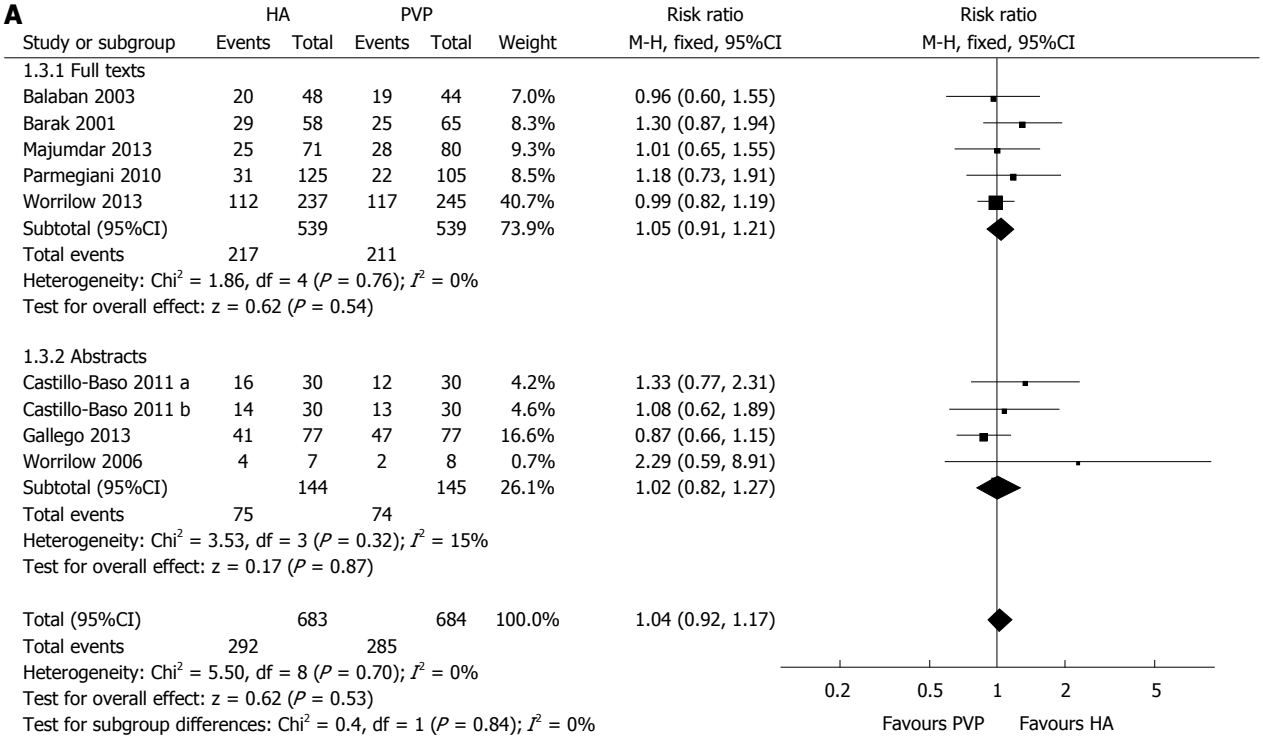


Figure 5 Secondary endpoints. A: The forest plot showing clinical pregnancy rate per transfer. Risk ratio is shown with 95%CI; B: The forest plot showing implantation rate per embryo transferred. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

Sub-group analysis considering the quality of the sperm, previous failed ICSI cycles, infertility cause, number and quality of transferred embryos should be considered in order to eliminate confounding factors.

Until then, this review may provide reassurance of non inferiority of one method over another for embryologists and laboratory staff involved in the acquisition of laboratory materials.

COMMENTS

Background

The majority of patients undergoing intracytoplasmic sperm injection (ICSI) treatment will reach the stage of embryo transfer due to important improvements of ovarian stimulation protocols and laboratory technology, but only a small proportion of transferred embryos implant leading to an overall success rate of 10%-40%.

Research frontiers

Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome.

Innovations and breakthroughs

Hyaluronic acid has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. Several studies investigated this method, but this is the first meta-analysis to assess the effect of using hyaluronic acid compared to PVP.

Applications

The results of this meta-analysis combining the outcomes from 11 randomised controlled trials concluded that there is no difference between hyaluronic acid and PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

Terminology

Sperm immobilisation and selection is an important step in the ICSI process and refers to the use of a medium in the laboratory for reducing the speed of sperm in order to allow its manipulation in the ICSI process.

Peer-review

The peer-reviewers appreciated the completeness of this meta-analysis. The manuscript was assessed as being well prepared, interesting, clear and well defined.

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