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Xiu-Xia Song, Director
Fang-Fang Ji, Vice Director
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Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
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FRONTIER

Latin American Dialysis and Transplant Registry: Experience and contributions to end-stage renal disease epidemiology

Ana Maria Cusumano, Guillermo Javier Rosa-Diez, Maria Carlota Gonzalez-Bedat

Ana Maria Cusumano, CEMIC University Institute School of Medicine, Buenos Aires, PC C1430EFA, Argentina

Guillermo Javier Rosa-Diez, Nephrology Service, Italian Hospital, Buenos Aires City, PC C1199ABC, Argentina

Maria Carlota Gonzalez-Bedat, Latin American Society of Nephrology and Hypertension, Montevideo City, PC 11600, Uruguay

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Correspondence to: Ana Maria Cusumano, MD, PhD, CEMIC University Institute School of Medicine, Valdenegro 4337, Buenos Aires, PC C1430EFA, Argentina. anacusumano@yahoo.com.ar
Telephone: +54-11-52990914

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Abstract

In 2015, 634387 million people (9% of the world's population) resided in Latin America (LA), with half of those populating Brazil and Mexico. The LA Dialysis and Transplant Registry was initiated in 1991, with the aim of collecting data on renal replacement therapy (RRT) from the 20 LA-affiliated countries. Since then, the Registry has revealed a trend of increasing prevalence and incidence of end-stage kidney disease on RRT, which is ongoing and is correlated with gross national income, life expectancy at birth, and percentage of population that is older than 65 years. In addition, the rate of kidney transplantation has increased yearly, with > 70% being performed from deceased donors. According to the numbers reported for 2013, the rates of prevalence, incidence and transplantation were (in patients per million population) 669, 149 and 19.4, respectively. Hemodialysis was the treatment of choice (90%), and 43% of the patients undergoing this treatment was located in Brazil; in contrast, peritoneal dialysis prevailed in Costa Rica, El Salvador and Guatemala. To date, the Registry remains the only source of RRT data available to healthcare authorities in many LA countries. It not only serves to promote knowledge regarding epidemiology of end-stage renal disease and the related RRT but also for training of nephrologists and renal researchers, to improve understanding and clinical application of dialysis and transplantation services. In LA, accessibility to RRT is still limited and it remains necessary to develop effective programs that will reduce risk factors, promote early diagnosis and treatment of chronic kidney disease, and strengthen transplantation programs.

Key words: Latin America; Chronic kidney disease; Renal replacement therapy; Kidney transplantation; Prevalence; Incidence; Epidemiologic registries; Risk factors

Core tip: In Latin America (LA), patients with end-stage renal disease on renal replacement therapy (RRT) are tracked by the LA Dialysis and Transplant Registry. Data from the Registry shows increasing prevalence and incidence, which are correlated with gross national income, life expectancy at birth, and percentage of population over 65 years. The Registry represents the only source of such data in many LA countries. Its contributions to the knowledge of RRT epidemiology in LA as well as to the education and training of nephrologists are highlighted in this article, and the need for its evolution towards population-based Registries is discussed.

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INTRODUCTION

Latin America (LA) is a conglomerate of nations sharing a common Latin ancestry and languages (mainly Spanish or Portuguese). It includes South and Central America, Mexico and the Hispanic Caribbean Islands. Multiethnic and multicultural, its population displays a great genetic diversity, resulting from multiple admixture events that occurred among the original European immigrants (mainly from Spain and Portugal but including other nationalities that arrived in large numbers during the so-called "Italian diaspora" in the late 19th and the early 20th century and also represented by those escaping the World Wars) and Native Americans (who currently represent the majority in Bolivia and very high percentages in Guatemala, Peru and Mexico) as well as the descendants of slaves that migrated from Africa (with very high numbers in Brazil in particular, and fewer in Colombia and Uruguay). The racial admixture is so robust that genetic studies arrived at the conclusion that physical appearance has poor reliability as an indicator of genetic ancestry for the LA peoples in general[1]. In Uruguay, specifically, it was reported, "the data show that almost every population is dihybrid or trihybrid, and when African influence is not detected, it is probably due more to the method than to an absence of that contribution"[2]. Thus, each Latin American country is considered to encompass its own unique ethnic characteristics.

The 2015 population estimate for LA is 634387 million (including Puerto Rico, a political territory of the Northern American-situated United States), accounting for roughly 9% of the global population[3]. Approximately one-half of the LA populations reside in Brazil and Mexico. Brazil, itself, is the biggest and most populous LA country and the 5th largest country in the world, both by geographical area and by population. LA annual growth is 1% per year, with 7.4% of its inhabitants being older than 65 years[3-5]. Approximately 8% of LA peoples identify themselves as indigenous, representing more than 522 groups dispersed broadly throughout the continent and speaking around 420 languages (*e.g.,* Quechua, Aymara, Guarani, among others)[6,7].

LA has experienced significant social and financial progress over the past decades; however, the improvements have happened in an inequitable way, contributing to striking disparities in health and economic conditions among social classes and geographic regions, between countries as well as within countries. As a result, LA is currently characterized by the Gini index (which describes how far away a country's income distribution is from complete equality) as having the highest socioeconomic inequality in the world. As a whole, however, socioeconomic indexes of LA countries have improved throughout the current century.

Gross national income (GNI) per capita (as calculated by the Atlas method) increased from an average of 3300 USD to 9941 USD between 2000 and 2014, but ranging from 830 USD in Haiti to 19210 USD in Puerto Rico[5]. Most of the LA countries have reached the status of upper middle income (4126 USD to 12735 USD), including Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Mexico, Panamá, Paraguay and Peru. Only one country - Haiti - qualifies as a low-income country (< 1045 USD). Five countries are included in the group of low middle income (1046 USD to 4125 USD; Bolivia, El Salvador, Guatemala, Honduras and Nicaragua), and five are included in the group of high income (> 12736 USD; Argentina, Chile, Puerto Rico, Uruguay and Venezuela)[8].

Life expectancy at birth has also shown an increasing trend in LA countries, from 70 years in 2000 to 75.5 years in 2015, approaching the observed indexes of more developed nations[3]. LA countries have seen increases in life expectancy, with Chile having the highest (80 years) and with Bolivia (67.8 years) and Haiti (62.6 years) having lower proportions of increase compared to the other countries. The overall improvement has been achieved largely through a reduction in child mortality. In contrast, the percentage of people living below the poverty line in LA countries continues to be high (28.1% in 2013), as does the percentage of people leaving under the line for extreme poverty (11.7%)[3]. Finally, the human development index (HDI; an indicator of the quality of life of a country, defined by the United Nations Development Program and which combines the three basic dimensions of life expectancy at birth, education and income per capita) is 0.748 for LA countries as a whole, but ranges from as low as 0.484 in Haiti to very high in Argentina (0.836) and Chile (0.832)[9].

Eighty percent of the populations of LA countries reside in urban areas, making LA the most urbanized region in the world[10]. The rate of urbanization ranges from 53.6% in Honduras to 91.8% in Argentina. Moreover, approximately 24% of LA inhabitants of

Table 1 Cardiovascular and renal risk factors, extracted from national surveys in 7 Latin American countries[1]

	Argentina	Brazil	Chile	Mexico	Uruguay	Ecuador	Paraguay
Survey year	2013	2013	2010	2012	2013	2013	2011
Hypertension	34.1	24.1	26.9	31.5	38.7	15.6	32.2
High cholesterol	29.8	20.3	38.5		23.9	24.5	25.5
Overweight	37.1	33.3	39.3	38.8	ND[1]	ND	34.8
Overweight + Ob	57.9	50.8	64.4	71.3	64.7	ND	57.6
Ob	20.8	17.5	25.1	32.4	ND	50	22.8
Diabetes	9.8	6.9	9.4	9.2	7.8	ND	9.7
Current smokers	25.1	11.3	40.6	19.9	28.8	25.9	14.5
Sedentarism	55.1	66.2	88.6		70	ND	74.5

[1]In which approximately 68% of the Latin American population resides. ND: Not detailed; Ob: Obesity.

large cities live in slum neighborhoods. More than 60 cities in LA have recorded populations of > 1 million inhabitants, including several cities that are characterized as megalopolis (defined as more than 10000000 people, including neighborhoods), such as San Pablo (Brazil), Mexico City (Mexico), and Buenos Aires City (Argentina)[3,10,11]. Over the past few decades, the population pyramid has changed its shape into that of a bell, reflecting its evolution towards that of an aging society in general. All of these features of the peoples residing in LA countries present new challenges to each country's healthcare system, which remain under the burden of ongoing challenges otherwise associated with lower socioeconomic regions, such as high rates of infectious diseases.

As in the rest of the world, chronic kidney disease (CKD) is prevalent throughout LA, due to both infectious disease prevalence and immature public health systems. Epidemiological information about the prevalence of early-stage CKD in the general LA population is scarce and of low quality. It is known, however, that CKD constitutes a higher burden in Central America, where a regional epidemic of CKD of unknown origin emerged during the last decade, affecting primarily young male agricultural workers from communities along the Pacific coast and southern México, especially sugarcane workers. In the involved areas, the national mortality rates have reached as high as 5 times the national rates, with El Salvador representing the country with the highest mortality rate from kidney disease worldwide[12,13].

Information about cardiovascular and renal risk factors is available through national health inquires in some countries; their results confirm that these risk factors are highly prevalent in LA, particularly in countries with greater percentage of inhabitants over 65-year-old, such as Argentina (11%), Brazil (7%), Chile (10%) and Uruguay (14%) (Table 1).

Renal replacement therapy in LA

Renal replacement therapy (RRT) started as peritoneal dialysis (PD) in Brazil in 1947. Shortly thereafter, the first hemodialysis (HD) was also accomplished in Brazil (in 1949), and the first kidney transplant in Argentina (in 1956)[14,15]. Initially, HD was considered exclusively as a therapy to support patients with acute renal failure and who were awaiting transplantation, but it quickly became incorporated as treatment for end-stage renal disease (ESRD). The field of clinical nephrology developed almost simultaneously, with physicians and researchers consolidating into National Nephrology Societies, such as those of Argentina (in 1960), Brazil (in 1960), Chile (in 1964) and Uruguay (in 1967).

The LA Nephrology and Hypertension Society [Sociedad Latinoamericana de Nefrología e Hipertensión (SLANH)] was created in 1970, grouping the various National Nephrology Societies of 20 countries (*i.e.*, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Uruguay and Venezuela). In 1991, the SLANH founded the LA Dialysis and Transplant Registry (LADTR) to promote the knowledge and improve the care of ESRD by collects and analyzing data from the 20 member countries. The Registry office was housed in Montevideo (Uruguay) until 2001, when it moved to Buenos Aires (Argentina), where it remained until 2012, when it returned to Montevideo. Currently, the Registry is composed of an Executive Board and delegates from each of the Nephrology Societies that are part of the SLANH.

The methodology used by the LADTR has been reported previously. Briefly, participant countries complete an annual survey to provide data on incident and prevalent cases of patients undergoing RRT by means of all modalities: HD, PD, and living with a functioning graft (LFG), CKD etiology, number and type of kidney transplants, percent of population under RRT coverage, and number of nephrologists, as well as other relevant parameters. Analyses of these variables are performed routinely to determine correlations with GNI and life expectancy at birth as well as other socioeconomic indexes[16-23].

Prevalence and incidence of ESRD under RRT

All 20 SLANH countries have and continue to participate in the annual surveys, providing data for > 90% of the LA populations since the beginning of the century. Table 2 describes the most prominent variables analyzed in

Table 2 Socioeconomic indexes, prevalence and incidence of renal replacement therapy and transplantation rate

Country	Population[1]	GNI	LEB	Prevalence rates, pmp					Incidence rate	Kidney Tx, *n*	Tx by deceased donors, %	Kidney Tx rate	Nephrologists, *n*	Nephrologists, pmp
				HD	PD	Total dialysis	LFG	Total RRT						
Argentina	42202935	13690	76	626.6	36.0	662.7	197.2	859.9	160.2	1287	68.4	30.5	1150	27.2
Bolivia	10448913	2220	67	195.2	18.3	213.5	31.6	245.1	94.8	75	24.0	7.2	24	2.3
Brazil	202740000	11640	74	449.6	45.6	495.2	212.6	707.8	180.3	5433	74.7	26.8	3300	16.3
Chile	17819054	14290	80	1019.1	61.2	1080.3	205.1	1285.4	182.4	234	74.8	13.1	132	7.4
Colombia	47661787	7020	74	349.0	143.6	492.6	111.3	603.9	89.7	680	99.7	14.3	95	2.0
Costa Rica	4773730	8850	80	42.3	76.0	118.4	282.6	400.9	ND	105	48.6	22.0	24	5.0
Cuba	11163934	6051	79	259.1	10.1	269.3	78.4	347.6	103.1	174	ND	15.6	524	46.9
Ecuador	16100000	3600	76	481.8	48.0	529.8	20.4	550.2	177.6	127	81.1	7.9	143	8.9
El Salvador	6401240	5360	72	232.5	288.7	521.1	73.6	594.7	390.1	20	0.0	3.1	47	7.3
Guatemala	16173133	3130	72	157.7	221.3	379.0	54.0	433.0	124.8	90	13.3	5.6	54	3.3
Honduras	8500000	2140	73	186.9	14.4	201.3	8.4	209.6	176.7	0	0.0	0.0	18	2.1
Jalisco (Mexico)	7742303	ND	ND	599.4	486.7	1086.1	567.4	1653.5	420.9	447	16.1	57.7	45	5.8
Nicaragua	6146000	1690	74	211.5	24.4	235.9	21.2	257.1	24.4	11	0.0	1.8	28	4.6
Panamá	3975404	9030	77	495.0	90.3	585.3	110.7	696.0	462.1	48	73.1	12.1	25	6.3
Paraguay	6783374	3310	75	165.7	4.0	169.7	19.9	189.6	20.2	26	79.0	3.8	46	6.8
Perú	30297279	5680	72	272.2	43.1	315.3	63.2	378.5	30.0	184	75.0	6.1	301	9.9
Puerto Rico	3615000	18370	79	1362.1	106.2	1468.3	378.4	1846.7	432.9	80	86.3	22.1	97	26.8
Rep Dominicana	12000000	5570	73	178.8	47.3	226.1	52.8	278.9	208.3	84	92.9	7.0	135	11.3
Uruguay	3406545	13670	77	692.2	71.6	763.8	323.5	1087.3	157.3	105	91.4	30.8	173	50.8
Venezuela	30389596	12460	74	505.1	0.0	505.1	60.8	565.9	ND	281	69.8	9.2	502	16.5
Totals	488340227	147771	75	442.0	67.0	509.0	159.0	669.0	149	9491	70.4	19.4	6863	14.0

[1]Most population data were provided by the participating countries; when not provided, data from the Latin American and Caribbean Demographic Centre-Population Division of Economic Commission for Latin America and the Caribbean were used instead. LEB: Life expectancy at birth; ND: Not detailed; Tx: Transplant.

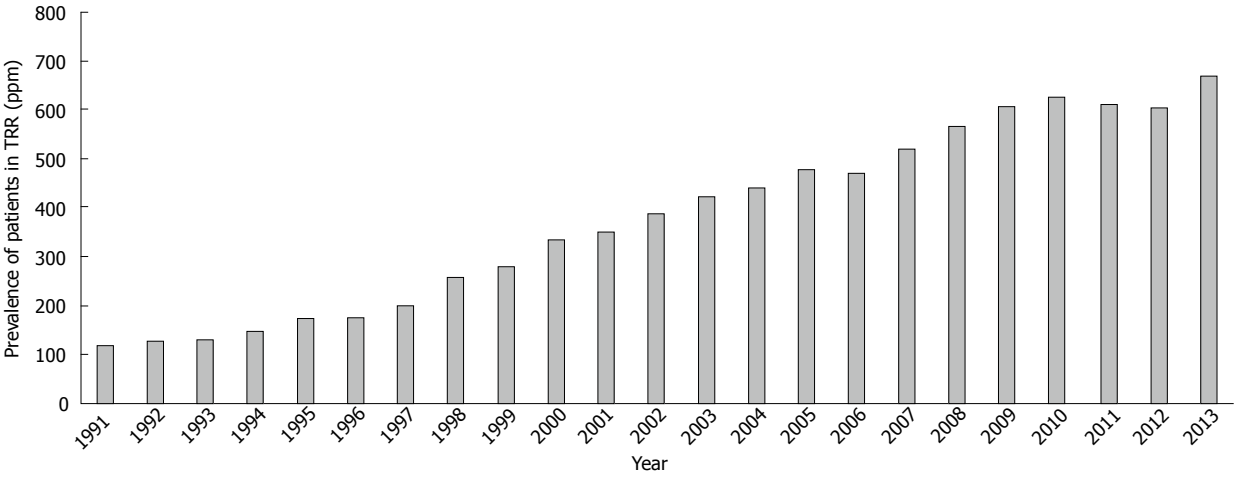

Figure 1 Prevalence of renal replacement therapy in Latin America, 1991-2013.

the last available survey.

From 1991 to 2013, the prevalence of ESRD under RRT increased from 119 patients per million population (pmp) to 660 (HD, 436 pmp; PD, 67 pmp; LFG 157 pmp) (Figures 1 and 2). Only six countries have RRT prevalence above the mean: Argentina, Brazil, Chile, Jalisco (Mexico), Puerto Rico and Uruguay, with the reported rates ranging from 778 pmp to 1847 pmp. The prevalence rates among the population over 65-year-old is particularly high, especially in the six countries accounting for the highest amounts of this population (Argentina, Brazil, Chile, Colombia, Puerto Rico and Paraguay) and reaching as high as 2400 pmp. As expected, the overall prevalence correlated with the percentage of people over 65-year-old (Figure 3). Moreover, every time it was analyzed, the prevalence correlated significantly with GNI and life expectancy at birth[16,19-21] (Figure 4).

An increase in RRT patients has occurred for all modalities, but HD in particular has proportionally increased more than PD and transplantation (Figure 2)[24]. HD continues to be the treatment of choice in the LA region (90%) and 43% of HD patients are located in Brazil. PD prevailed in 2013 only in Costa Rica (64.2%), El Salvador (55.4%) and Guatemala (58.4%). PD was also common in Colombia, although the percentage of

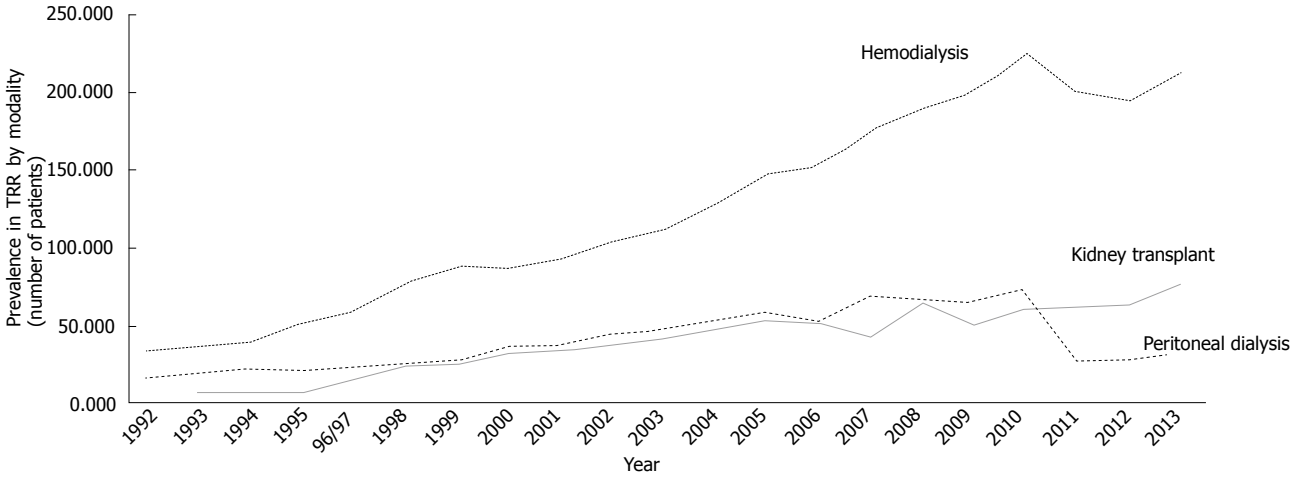

Figure 2 Prevalence of renal replacement therapy in Latin America by modality.

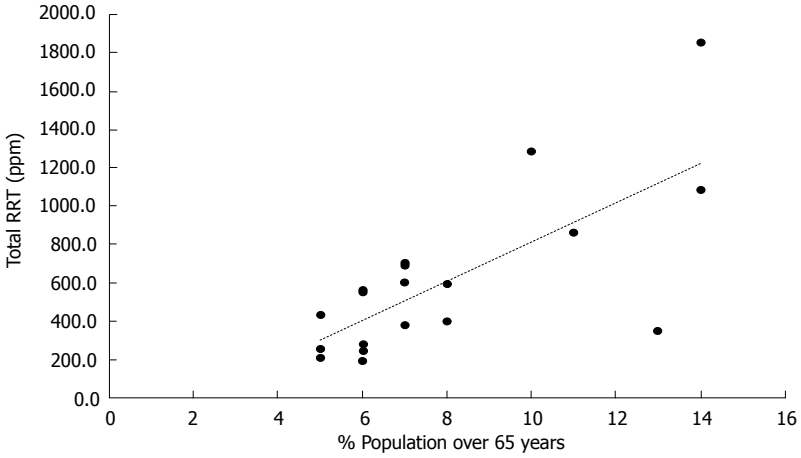

Figure 3 Correlation between renal replacement therapy prevalence and percentage of population over 65-year-old.

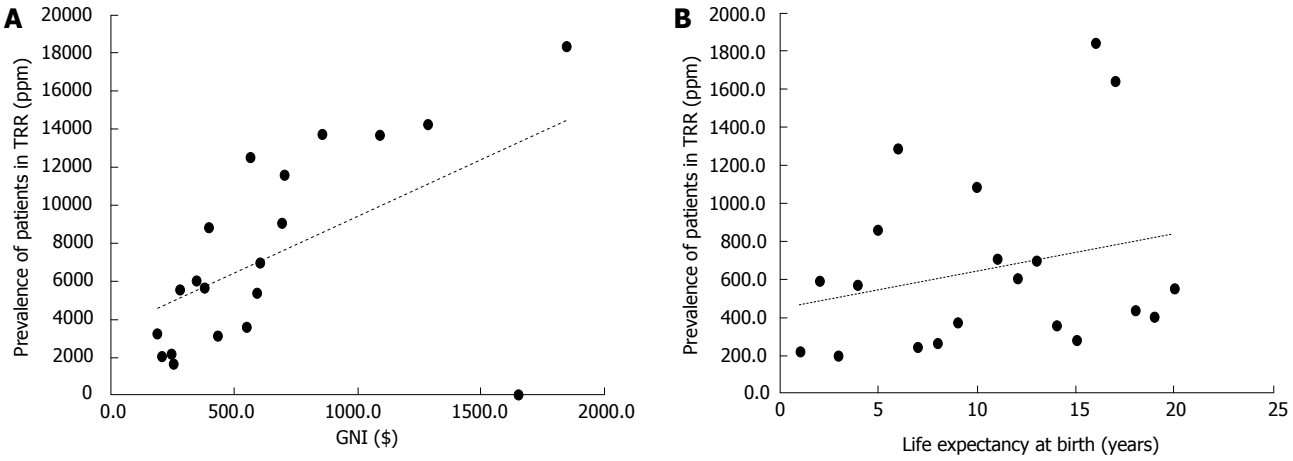

Figure 4 Renal replacement therapy prevalence and correlation with gross national product and life expectancy at birth. A: For gross national product (GNI), the correlation coefficient is 0.43; B: For life expectancy at birth, the correlation coefficient is 0.34.

Colombian PD patients has consistently decreased over the last years (from 54% in 2000 to 29.2% in 2013)[24].

Data for incidence of RRT were provided to the LADTR by 18/20 countries in 2013, accounting for 92.7% of the population of LA. A wide rate variation was observed, from 462.1 pmp in Panama to 20 in Paraguay. A tendency towards rate stabilization/little growth was found for most countries, except in the Central America countries of El Salvador, Guatemala, Honduras and Panama, which showed a significant increase in incidence. Diabetes continues to be the leading indication for dialysis. The LADTR received data on RRT incident

Table 3 Total incidence rates and percentages of new diabetic renal replacement therapy patients

Country	Population	Incident patients, *n*	Incident rate	Diabetics, %
Argentina	42202935	6760	160.2	35.1
Bolivia	10448913	991	94.8	30.0
Brazil	202740000	36548	180.3	40.0
Chile	17819054	3250	182.4	16.7
Colombia	47661787	4274	89.7	33.5
Ecuador	16100000	2860	177.6	30.0
Guatemala	16173133	2018	124.8	30.0
Jalisco (Mexico)	7742303	3259	420.9	58.0
Nicaragua	6146000	150	24.4	41.6
Paraguay	6783374	137	20.2	45.3
Peru	30297279	910	30.0	32.2
Puerto Rico	3615000	1565	432.9	66.9
Uruguay	3406545	536	157.3	27.7

diabetic patients from 13 countries, accounting for 84.2% of the entire LA population. The reported percentage of diabetic patients from each country ranged from 16.7% to 66.9% (mean, 37.5%). The highest diabetes incidence rates were reported by Puerto Rico (66.9%), Jalisco (Mexico) (58%) and Paraguay (45.3%), while the lowest rates were reported by Uruguay (27.7%) and Chile (16.7%) (Table 3). The overall RRT incident diabetics rate was 58 pmp, lower than that reported from the United States Renal Data System in the same year (158.4 pmp), but more than double that from the European ERA/EDTA Registry (24 pmp)[25,26]. In addition, the LADTR indicated that 19.4% of the RRT population was placed on a kidney transplant waiting list.

When compared with the United States data from 2013, incidence in LA, as a whole, was substantially lower (149 *vs* 363)[25], but when looking individual countries, Jalisco (representing Mexico), Puerto Rico, Panama and El Salvador have similar rates (421, 433, 462 and 390, respectively). When compared to the European ERA/EDTA registry (112 pmp)[26], the rate is higher in most LA countries (Table 2). Probably, there is more than one reason for the striking differences in incidence in LA, such as higher prevalence of diabetes mellitus (in Mexico and Puerto Rico, in particular) (Table 2) or of CKD of unknown origin (termed as Mesoamerican Nephropathy, involving the Pacific coast and southern Mexico)[12,13], or varying access to RRT among the countries (Nicaragua and Paraguay). Overall, LA prevalence rates (660 pmp) are very far from those reported for the United States (2014 pmp) and are closer to those reported from the European ERA/EDTA (738 pmp)[24,25].

The data in the LADTR indicates that the overall kidney transplant rate increased from 3.7 pmp in 1987 to 6.9 in 1991 and then to 19.4 in 2013; although, there were remarkable disparities among the various countries in the last year: 57.7 pmp in Jalisco (Mexico), 32 pmp in Uruguay and 1.8 pmp in Nicaragua. The highest number of transplants (*n* = 5433) occurred in Brazil, which had a transplant rate of 26.8 pmp for 2013. A total of 244 double kidney-pancreas transplants were performed in LA in 2013: Brazil, *n* = 120; Argentina, *n* = 63; Costa Rica, *n* = 51; Colombia, *n* = 5; Uruguay, *n* = 3; Ecuador, *n* = 1; Chile, *n* = 1. The total number of all kidney-related transplants in 2013 was 9491, with 70.4% from cadaveric donors; the highest percentages of the latter were reported by Colombia (99.7%), Dominican Republic (92.9%) and Uruguay (91.4%).

Even though kidney transplantation is feasible, available, and an increasingly used modality for RRT in all LA countries, its growth rate is not as fast as it should be in order to compensate for the increased prevalence of patients on waiting lists for transplantation. This fact further strengthens the need to implement transplant programs and procurement of suitable organs. Moreover, the key issues identified in this study - specifically, the increased incidence of patients in RRT for all modalities in the LA region, and diabetes continuing to be the leading clinical cause for RRT - highlight the crucial nature of prevention programs for CKD to achieve early diagnosis and treatment. Yet, there is a wide gap in the amount of nephrologists among each LA country (from 2 pmp in Colombia to 50.8 pmp in Uruguay) that must be taken into consideration.

Contributions for improvement of nephrology and ESRD knowledge and care in LA

Since its creation, the LADTR has fulfilled its mission, providing valuable information on epidemiology and burden of ESRD under RRT in the region and correlating the data with socioeconomic indexes. The results of the LADTR annual surveys have been published, providing consistent data and trends about ESRD under RRT and national variations inside the region, thereby transforming the registry into a powerful tool for health authorities and highlighting the necessity of guaranteeing full access to RRT while establishing transplantation and procurement programs[16-24]. In addition, the observation that the primary etiologies of ESRD are preventable (*i.e.,* diabetes and kidney hypertensive disease) has prompted the development of National Clinical Practice Guidelines

for CKD Diagnosis and Treatment and the creation of programs aimed at accomplishing early detection and treatment. Recently, based upon the work of the LADTR, the Pan American Health Organization (PAHO) included in its Strategic Plan a specific target for ESRD treatment - namely, to reach, by the year 2019, a RRT prevalence of, at least, 700 pmp for all ESRD cases in all LA countries[27]. To achieve this objective, the PAHO estimates at least 20 nephrologists pmp will be needed in each country.

Moreover, the LADTR, through its participation in National, LA, Hispanic American and International Congresses and Meetings and in the International Society of Nephrology Kidney Health in Disadvantaged Populations Committee, has contributed to spreading knowledge of the epidemiology of RRT throughout the nephrology community in the LA region, and to enable productive comparisons with the rest of the world. The LADTR has also stimulated the development of voluntary and obligatory national renal registries. At present, 10 countries have consolidated national registries: Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Puerto Rico, Paraguay, Uruguay and Venezuela; some of these were initiated in the 1980s, such as the Chilean (1980) and the Uruguayan (1981) ones, so that they are providing a rich database of information today[28].

The LADTR has simultaneously improved the training of nephrologists in epidemiology, having organized, in conjunction with the European ERA/EDTA Registry, the Introductory Course in Epidemiology in Buenos Aires (2007, Argentina), Mexico City (2009, Mexico) and Cartagena de Indias (2011, Colombia). It is appropriate to recognize, here, the permanent collaboration of the Spanish Society of Nephrology, in particular its fellowship program for Latin Americans, which has allowed young nephrologists to train abroad.

The continuity of the LADTR along the years, whose members participate without any fee, has implied a sustained effort of the entire Latin American nephrology community.

Future of the LADTR

The LADTR recognizes several limitations. In most Latin American countries, reporting to the registry is not mandatory, not all the countries report all the data each year, data are collected from each country on a global basis, and, in the past, data from some province or region have been extrapolated to the whole country. This last feature was the case for Mexico, wherein prevalence and incidence were extrapolated from the data of the Mexican states of Jalisco and Morelos until the year 2010; moreover, since 2011, only the data from Jalisco has been deposited in the LADTR. Furthermore, the LADTR cannot report survival data for any of its participating countries, as it currently collects only aggregated data. Finally, the number of LFG patients in many countries is estimated and not definitive.

In spite of its limitations, the LADTR has provided current knowledge about trends of RRT prevalence and incidence in a defined geographical zone (LA) and in defined countries (the 20 that comprise the SLANH), has shown that, in part, the increase in incidence is related to the expansion of the burden of kidney diabetic disease, and has revealed the striking differences in prevalence and incidence that are associated with the countries' wealth status and health coverage.

Heterogeneity or even the absence of registries in some Latin American countries is congruent with the inequities in access to RRT in such countries, as well as the limited availability of skilled human resources. The inclusion by the PAHO Strategic Plan of a goal of 700 pmp under RRT by 2019 undoubtedly will contribute to increased health coverage and the implementation of obligatory national registries, all of which, added to the sustained contribution of the nephrology community, will undoubtedly result in improved quality of national-based registries and the LADTR itself, supporting the development of population-based registries.

The LADTR has sustained its continuity over the years and, at present, it is the only source of data about RRT in the region and for many of its member countries. Its future depends on the quality of its data, which in turn depends on the data provided by the respective national registries and their (and its) quality control procedures. To continue its tasks, in the future, the LADTR will likely require funding that is sufficient to strengthen quality-controlled data.

CONCLUSION

Since its creation in 1991, the LADTR has provided valuable information on epidemiology and burden of ESRD under RRT and continues to be, at present, the only source of RRT data for this region. Prevalence and incidence of RRT continue to increase throughout LA. Prevalence correlates with GNI, life expectancy at birth and percentage of the population over 65-year-old. In the LA region, as a whole, it is still necessary to increase full accessibility to RRT and to develop programs that will facilitate better control of risk factors and early diagnosis and treatment of CKD, as well as implementation of effective transplantation programs.

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REVIEW

Vascular calcification: When should we interfere in chronic kidney disease patients and how?

Usama Abdel Azim Sharaf El Din, Mona Mansour Salem, Dina Ossama Abdulazim

Usama Abdel Azim Sharaf El Din, Department of Nephrology, School of Medicine, Cairo University, Cairo 11759, Egypt

Mona Mansour Salem, Department of Endocrinology, School of Medicine, Cairo University, Cairo 11759, Egypt

Dina Ossama Abdulazim, Department of Rheumatology and Rehabilitation, School of Medicine, Cairo University, Cairo 11759, Egypt

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Correspondence to: Usama Abdel Azim Sharaf El Din, MD, Professor, Department of Nephrology, School of Medicine, Cairo University, 58th Abbas El Akkad St., Nasr City, Cairo 11759, Egypt. usamaaas@gmail.com
Telephone: +20-11-11333800
Fax: +20-22-22753890

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Abstract

Chronic kidney disease (CKD) patients are endangered with the highest mortality rate compared to other chronic diseases. Cardiovascular events account for up to 60% of the fatalities. Cardiovascular calcifications affect most of the CKD patients. Most of this calcification is related to disturbed renal phosphate handling. Fibroblast growth factor 23 and klotho deficiency were incriminated in the pathogenesis of vascular calcification through different mechanisms including their effects on endothelium and arterial wall smooth muscle cells. In addition, deficient klotho gene expression, a constant feature of CKD, promotes vascular pathology and shares in progression of the CKD. The role of gut in the etio-pathogenesis of systemic inflammation and vascular calcification is a newly discovered mechanism. This review will cover the medical history, prevalence, pathogenesis, clinical relevance, different tools used to diagnose, the ideal timing to prevent or to withhold the progression of vascular calcification and the different medications and medical procedures that can help to prolong the survival of CKD patients.

Key words: Chronic kidney disease; Uremia; Calcification; Sevelamer; Calcific uremic arteriolopathy; Fibroblast growth factor 23; Klotho; Phosphate binders; Kidney transplantation

Core tip: The last 2 decades witnessed the failure of all intervention studies targeting different risk factors of vascular calcification in chronic kidney disease (CKD) patients on regular hemodialysis. The main aim of all these studies was to decrease cardiovascular morbidity and mortality among such patients. These disappointing results criticized the value of such interventions in clinical practice. On the other hand, when similar trials were run on patients at an earlier stage of CKD, most of these

trials showed a significant impact on patient survival and/or cardiovascular morbidity. Such discrepancy indicates the value of timing of interference. We are trying in this review to develop the ideal strategy that would optimize the management of CKD patients to avoid the devastating vascular calcification, highlighting the value of different medicines used in this plan. Meanwhile we are showing the update in guidelines concerned with this issue.

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INTRODUCTION

Vascular calcification (VC) affects either the arterial tree or cardiac valves. Deposits of hydroxyapatite in the arterial wall occur within either tunica intima or tunica media. VC is a strong predictor of increased cardiovascular mortality among chronic kidney disease (CKD) patients. However, the different clinical studies that tried to manipulate the different risk factors of VC in dialysis patients failed to show a significant impact on patient survival. On the other hand, when pre-dialysis patients underwent similar studies, there was a significant decrease of cardiovascular and overall mortality rates, beside a comparable effect on vascular calcification progress rate. These results have probably two explanations. Dialysis patients might have advanced VC rendering their arteries permanently and irreversibly damaged [approaching end stage arterial damage (ESAD[1])] or they might have additional pathologic problems exceeding in their survival impact the VC The authors of this review are inclined towards the 1st possibility and will try to outline the best way to tackle this devastating pathology.

HISTORY

In 1855, "metastatic calcification" was described in three patients with renal disease[2]. Eight years later, Virchow[3] reported that this calcification is a definite ossification.

The first reported VC in infants was probably that of Durante[4] describing aortic and pulmonary artery calcification. Calcification of peripheral vessels has been described in a few children with CKD as early as 1942[5,6].

VC affected humans in ancient history. The most ancient calcification so far reported is 5000 years ago, "identified in the recently discovered ice man"[7]. On the other hand, the earliest coronary calcification reported is that of an Egyptian mummy who was living 4000 years ago[8].

VC IN CKD PATIENTS

A high percentage of CKD patients show VC The prevalence among predialysis CKD G3-5 patients was 79% of cases in one study[9]. It might approach 100% in patients starting dialysis[10]. VC significantly contributes to morbidity and mortality of CKD[11-13]. Up to 3- to 4-fold increase in VC has been reported in the earliest phases of CKD[14].

CKD patients show VC in almost all arteries whether large, medium or small-sized vessels, including the coronary arteries[10,15-17]. VC can affect the tunica intima and/or the tunica media of the arterial wall[18]. Intimal calcification is mainly a feature of atherosclerosis[19]. CKD patients can have intimal and medial calcification. Medial calcification is reported in CKD of any age[20,21]. When epigastric arteries of patients with end-stage renal disease (ESRD) were examined at the time of kidney transplantation, vessel calcification was detected regardless of patient age and/or the presence of other risk factors for atherosclerosis[22]. In one study, hemodialysis (HD) patients have higher calcification scores than either peritoneal dialysis (PD) or CKD G4. More heavily calcified patients were significantly older and mostly male[23]. In HD patients, coronary calcification progresses steadily[24]. High serum phosphate concentration was a strong independent risk factor only in non-diabetic patients. Diabetic patients lack similar association[25].

Fifty percent of CKD patients die out of cardiovascular events[26]. Cardiovascular mortality is 20- to 30-times of controls matching in age, race and gender[27]. Patients starting dialysis at age of 25-29 years have a median life expectancy of 18.5 years. This means that their survival is 33 years less than normal personnel[28]. Arterial calcification is one of the predictors of this increased cardiovascular mortality[28]. Patients with CKD should, therefore, receive aggressive preventive measures to reduce this cardiovascular disaster[29].

Coronary artery calcification (CAC) is common among CKD patients whether adolescents, adults or old aged, starting in early stages of CKD and steadily progressing in HD patients[30-32]. Postmortem study of atheromatous lesions in ESRD patients found more intense calcification of such lesions compared to age- and sex- matched controls[33].

Calcification of the internal iliac arteries in CKD patients was greater compared with controls[34].

Large vessel disease is associated with decreased arterial compliance as detected by ultrasound and accounts for the increased mortality[35,36].

Calcific uremic arteriolopathy (CUA), also called calciphylaxis is an obliterative vasculopathy affecting cutaneous arterioles. It occurs almost exclusively in ESRD patients. Affected arterioles show medial calcification[37]. Ischemia and necrosis of the skin, subcutaneous fat, visceral organs and skeletal muscles eventually ensues. The skin

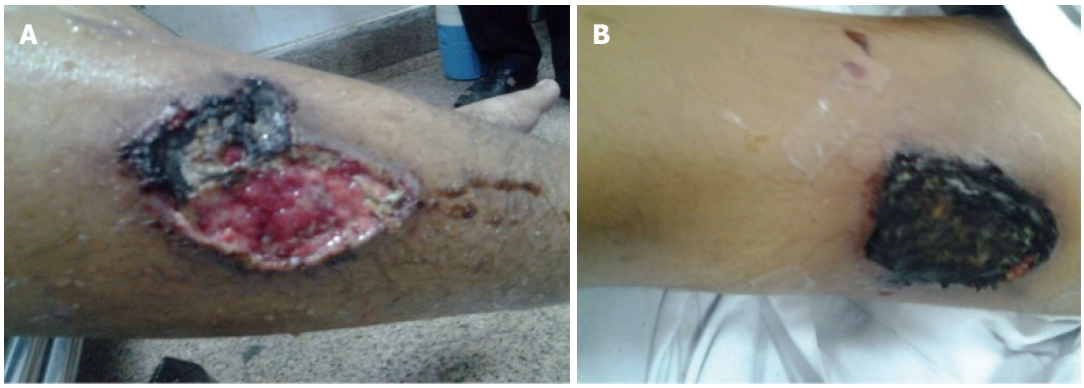

Figure 1 Male patient, 36-year-old, on regular hemodialysis for 8 years, presenting with multiple skin ulcers affecting both legs. A: His corrected serum calcium is 10.28 mg/dL and serum phosphorus 8 mg/dL. Serum PTH is 2588 pg/mL. He initially experienced itching papules that eventually ulcerated; B: Another ulcer with necrotic floor in the same patient. PTH: Parathyroid hormone.

manifests by necrotic foci and painful ulcers (Figure 1)[38].

VC IN KIDNEY TRANSPLANT RECIPIENTS

Death with a functioning graft is one of the major causes of graft loss (accounting for 42% of graft loss) in kidney transplant recipients (KTRs). Cardiovascular events are the first cause of death in this population affecting 36% to 55% of patients. The impact of VC on morbidity and mortality of KTRs is not appreciated enough[39-41]. Three point five percent to five percent of KTRs experience fatal or non-fatal cardiovascular events annually. This rate is much higher than in the general population. The prevalence of coronary artery calcification (CAC) in KTRs is higher (61%-75%) than that assessed in stage 3 CKD[42-44] and lower than that found in HD patients[45]. Moe *et al*[37] did not observe CAC progression after a successful kidney transplant. On the other hand, Oschatz *et al*[46] observed a significant progression within the first 6 mo, but no significant change between months 6 and 12 after a kidney transplant. All these trials were short term. When longer-term follow-up trials were performed, kidney transplant was found to favorably affects but does not halt CAC progression, with an annual rate of CAC progression ranging between 11% and 12.5%[47-49]. The risk of progression was higher in Caucasian race, with increased body mass index, higher baseline CAC score, higher diastolic blood pressure and lower glomerular filtration rate 3 mo after transplantation[50]. Other risk factors included inflammation, hyperparathyroidism and dialysis duration[47,51,52]. CAC score was significantly lower in KTR who had a pre-emptive transplant in comparison to those who underwent dialysis before transplantation (3.7 *vs* 102.9, $P < 0.001$)[52]. According to these studies, it seems that pre-emptive kidney transplant gives ESRD patients their best chance to avoid progressive VC.

PATHOGENESIS OF VC

Many factors summate the pathogenesis of VC in CKD. Such factors are either traditional or CKD related. The factors related to CKD include high serum calcium and phosphorus, increased dialysis vintage, increased duration of uremia[53], low serum fetuin-A level[53], and high serum level of fibroblast growth factor 23 (FGF23)[10,54-63]. Dialysis vintage, disturbed mineral metabolism and FGF23 are the most relevant factors having impact in the VC of CKD[37]. There is an association between VC and indices of low bone turnover in dialysis patients[64].

Is VC an active process?

More than 150 years ago, Virchow[2] was the first to report that vascular calcium deposits were real ossification. In CUA, vascular smooth muscle cells express osteopontin, bone sialoprotein, and osteonectin[37,65]. In non-calcified arteries in the same skin biopsy section, osteopontin or other bone proteins were not observed[65]. It seems that the deposition of these proteins predispose calcification[37,66].

Role of phosphorus

Vascular smooth muscle cells and osteoblasts originate from the same mesenchymal cell. Core binding factor α-1 (Cbfa1) turns the mesenchymal cell into osteoblast[37,67]. β-glycerophosphate is a phosphate donor. Vascular smooth muscle cells mineralize in the presence of this phosphate donor and increased Cbfa1 activation[37,68]. Calcific arterial lesions in patients devoid of CKD showed increased expression of Cbfa1 while normal arteries failed to show similar finding[37,69]. The findings of Cbfa1 in both CKD vascular lesions and non-CKD arterial disease might denote a common pathogenesis of VC. A significant relationship between increased serum phosphorus and obstructive atherosclerotic coronary artery disease was observed in non-CKD patients[37,70,71].

Bone morphogenetic protein-2

When bovine vascular smooth muscle cells (BVSMCs) were incubated in uremic serum and healthy control serum, upregulation of Cbfa1 was significantly higher with uremic serum. When β-glycerophosphate was added to increase the inorganic phosphorus within culture media, Cbfa1 significantly increased in normal control serum culture and

the significant difference in Cbfa1 was muffled[72]. This increase in Cbfa1 was completely inhibited after addition of foscarnet (an inhibitor of sodium/phosphate co-transport) to the normal serum. In case of uremic serum, inhibition was partial, denoting other factors might have an action on Cbfa1 beside hyperphosphatemia[37]. Bone morphogenic protein-2 (BMP-2) concentration is doubled in CKD serum. BMP-2 was detected in human calcified arteries[37,73-75] and human uremic serum can induce *in vitro* calcification that increases as the CKD advances[37,76].

Fibroblast growth factor 23 - klotho axis

Fibroblast growth factor 23 (FGF23) was isolated 16 years ago[77]. FGF23 is responsible for autosomal dominant hypophosphataemic rickets (ADHR) in humans[78] and is the humoral factor secreted by tumors inducing hypophosphatemia and osteomalacia (TIO)[79]. FGF23 plays an important role in the regulation of serum phosphate level. FGF23 is secreted by osteocytes in bone[80]. Other sites might share in FGF23 synthesis, including bone marrow, thalamus, lymph nodes and thymus[81]. The serum levels of FGF23 are derived mainly from bone[82]. FGF23 exerts its hypophosphatemic effect through inhibition of phosphate reabsorption by proximal tubular epithelial cells. It down-regulates the luminal sodium-phosphate co-transporters. FGF23 also inhibits 1α hydroxylase[83]. It was not clear if FGF23 stimulates secretion of parathyroid hormone (PTH)[82] or PTH stimulates FGF23 secretion. Klotho acts as a co-receptor for FGF23 by markedly increasing the affinity of FGF23 for ubiquitous FGF receptors (FGFR)[84]. Klotho, is highly expressed in the kidney and the parathyroid glands[84,85].

Klotho is an anti-senescence protein[86]. It exists in 2 forms: The transmembrane and the soluble secreted form[87,88]. Klotho is detected as soluble protein in body fluids including blood, urine[89-91] and cerebrospinal fluid[89].

The highest expression of Klotho is in kidney and brain[86,90,91], but it is also expressed in parathyroid gland[92,93] and heart[94] with less abundance.

The similarity of the phenotypes between $Kl^{-/-}$ mice[86] and $Fgf23^{-/-}$ mice is striking[95], which strongly suggests a common signaling pathway shared by these molecules[96,97]. Now it is well documented that membrane Klotho functions as the coreceptor for FGF23, which amplifies and confers specificity of FGF23 action[84,85,98,99].

In contrast, soluble Klotho protein functions independently of FGF23[91] and plays an important role in modulation of ion transporters or channels[91,100], antioxidation[101] and anti senescence[102,103], in addition to simply supporting FGF23 action[104]. The protective effect of Klotho against soft tissue calcification is mediated by at least 3 mechanisms: Increasing urine phosphate excretion, renal protection and inhibition of phosphate uptake by vascular smooth muscle cells (VSMCs) and their dedifferentiation[104].

Klotho and FGF23 are likely responsible for calcium and phosphate homeostasis[105,106]. *In vitro* PTH secretion and mRNA transcription are inhibited by FGF23[107]. On the contrary, primary hyperparathyroidism in rodents is associated with increased FGF23 levels that are reduced by parathyroidectomy. PTH stimulates osteocytes to secrete FGF23[108]. In physiological settings in which there are normal Klotho and FGFR expression, FGF23 decreases PTH production, increases expression of both the parathyroid Ca-sensing receptor and the vitamin D receptor, and decreases cell proliferation[92].

In Klotho mutant mice, the different pathologic manifestations could be reversed when deficient Klotho is replaced[109-111]. Exogenous klotho was found to ameliorate kidney injury and renal fibrosis in a rat model of CKD[112]. It can also ameliorate endothelial cell senescence and muffles the binding of NFκB to nuclear DNA[113].

Patients with stages 3b-5 CKD and dialysis patients often develop high serum FGF23[114]. This elevation can even occur as early as stage 2 CKD, long before any changes in calcium, phosphate, or PTH are apparent[115]. Elevation in FGF23 stimulates the excretion of phosphorus by surviving nephrons. This would prevent the early onset of hyperphosphatemia in spite of increased bone turnover and the progressive decline in functioning nephrons. Development of CKD is associated with significant decline of Klotho mRNA expression[116]. This deficiency might explain the increased serum FGF23 levels in CKD as a result of end-organ resistance to the action of FGF23. By the time the patients reach ESRD, FGF-23 concentrations are often 100- to 1000- fold above the normal range[117], and moreover, circulating FGF-23 in ESRD patients is mostly intact and biologically active[118]. Three possible explanations could account for such elevation. First, increased secretion into and decreased removal of FGF23 from the circulation. Treatment with corticosteroids could activate osteocytes in pediatric CKD patients, and then significantly stimulate FGF-23 synthesis[119]. FGF-23 levels and estimated glomerular filtration rate (eGFR) were inversely correlating among individuals with CKD stage G4-5[120]. Second, the other cause of increased levels of FGF-23 may be related to decreased klotho and end organ resistance to FGF23 action in CKD[121]. Treatment of CKD patients with vitamin D may be the third cause. In 5/6 nephrectomized rats, intravenous administration of 1,25-(OH)2D, three times a week increased serum FGF-23[122].

The first report of a positive correlation between FGF23 and VC among HD patients was 6 years ago[10]. Similar results were reported in cases with CKD stages 2-5D. Patients with higher aortic and coronary calcification scores had elevated FGF23 levels[62]. Similar results were found in healthy older men irrespective of traditional risk factors[123]. Pediatric studies confirmed the same results in children with CKD[124]. The same association was recorded in patients kept on HD for more than one year[125].

Klotho deficiency in CKD vessels likely potentiates the development of accelerated calcification[126]. Restoration of Klotho and FGFRs by vitamin D receptor activators

renders human vascular smooth muscle cells FGF23-responsive, and that may be the mechanism of their anti calcific effects[126].

Increased FGF23 level is associated with increased risk for mortality among incident HD patients, during their first year of treatment[127]. This association was also confirmed in prevalent dialysis patients[128]. Neutralization of FGF23 in CKD rats was found to accelerate VC and increases mortality[129].

Inflammation

Atherosclerosis and VC accelerate in states of chronic inflammation. The later is one of the hallmarks of uremia. Uremic status was incriminated in the pathogenesis of chronic inflammation, however, the exact pathogenesis was not fully understood. Altered gut microbiome might affect the integrity of the intestinal barrier leading to facilitated blood translocation of bacteria and uremic toxins[130]. Inflammation also results from multiple co-morbid conditions activating inflammation (like infections and autoimmune systemic diseases)[131]. Many of the inflammatory markers and mediators are found to promote VC in CKD patients. These factors include interleukin 1 (IL-1), IL-6, C-reactive protein and tumor necrosis factor alpha (TNFα)[132-137].

The association between FGF-23 and vascular calcification was mitigated when corrected for inflammation markers[138]. In spite of this important role of inflammation that might underlie the role of Klotho-FGF23 axis, no intervention studies to target inflammation to prevent or stop VC progression in CKD were done.

Inhibitors of vascular calcification

All CKD patients are exposed to the uremic environment, however, not all of them will develop VC, suggesting that protective mechanisms also exist[139].

Fetuin-A inhibits precipitation of calcium-phosphate[140]. Fetuin-A synthesis is mainly hepatic. Its serum concentration falls with activation of cell mediated immunity[141]. Fetuin-A calcium phosphate complex is called calciprotein particles (CPP). In comparison to hydroxyapatite, CPP induce significantly less cytokine secretion when macrophages are exposed to equimolar concentrations of hydroxyapatite and CPP[142]. Mice deficient in fetuin-A develop extensive renal, myocardial, pulmonary, lingual and cutaneous calcifications[140]. CKD patients with fetuin-A deficiency develop increased cardiovascular mortality[140].

Matrix GIa protein (MGP) is a vitamin K dependent protein, synthesized in the bone[143]. MGP has an inhibitory role in VC[144,145]. MGP inhibits the formation of calcium crystal[73]. CKD is associated with decreased uncarboxylated MGP level with subsequent increased rate of VC and atherosclerosis[146].

Osteoprotegerin (OPG) is another anti-calcific agent. High OPG level is reported in patients with vascular calcification[147,148]. Increase in OPG level may be a self-defensive mechanism against factors promoting VC[148].

Vitamin K likely prevents post-menopausal fractures[149]. Vitamin K deficiency increases the chance of severe aortic calcification[150]. Treatment of rodent with vitamin K2 reduced VC[151]. Treatment of HD patients with vitamin K increases serum MPG and osteocalcin levels[140]. Dietary menaquinone might be more effective compared to phylloquinone, in prevention of the progression of vascular calcification. Studies linking vitamin K status to calcification outcomes in CKD are needed to determine the therapeutic value in such cases[152].

Pyrophosphate (PPi) directly blocks hydroxyapatite formation. PPi is synthesized in VSMCs[153]. PPi deficiency results in excessive arterial calcification[154]. Plasma PPi is deficient in HD patients, and is negatively correlating with VC[155,156].

Vitamin D deficient mice develop excessive VC[157]. Vitamin D deficiency is frequent among CKD patients. Decreased dietary intake, decreased synthesis in the skin and decreased 1α-hydroxylase activity in the failing kidney are the main causes. Further inhibition of 1α-hydroxylase ensues when serum FGF23 rises[158]. In CKD G 3-4, CAC was elevated in both the mild and severe vitamin D deficient cases[159]. Serum levels of 25(OH)D is negatively associated with VC in CKD G4-5[160]. Low plasma level of 25-hydroxy - vitamin D is associated with increased mortality in different stages of CKD. Progression to ESRD was accelerated in vitamin D deficient patients[161-163]. At therapeutic dosages sufficient to correct secondary hyperparathyroidism, VDR activator (VDRA) treatment of mouse model of CKD protected the vasculature from calcifying, but higher doses stimulated aortic calcification[164]. The latter was probably caused by indirect, endocrine VDRA effects resulting in hyperphosphatemia and hypercalcemia. Organ cultures of human arteries from patients with CKD exhibited significant upregulation of Klotho mRNA levels following 48 h of calcitriol or paricalcitol treatment. This treatment effect was not observed in arteries from healthy individuals. Therapeutic dosages of VDRA were also found to reduce VSMC phenotype transformation in the aorta[124].

To sum up, it seems clear that VC is triggered by different promoting factors that increase in CKD together with the deficiency of different protective factors. In other words, VC in CKD patients is the result of the interaction of this collection of offenders and inhibitors[165].

CLINICAL RELEVANCE OF VC

Sudden cardiac death, arrhythmia, congestive heart failure, or stroke are the major causes of death in patients with VC[166,167]. Most of the data on prognostic value of VC are extrapolated from studies in patients with normal kidney function. CKD patients sill need prospective clinical trials evaluating the prognostic impact of aortic, coronary and carotid calcification in different CKD stages[168]. The European Renal Best Practice (ERBP) work group recommends screening of incident dialysis patients[169], whereas some national guidelines dictated the screening of any CKD patient[170]. KDIGO guidelines, issued during 2009, considered that patients with CKD stages 3-5D and with known VC as

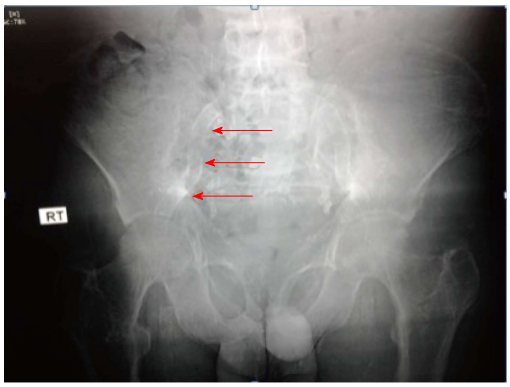

Figure 2 Plain X-ray of the pelvis in hemodialysis patient for 52 mo showing extensive calcification of the right common and external iliac arteries (arrows).

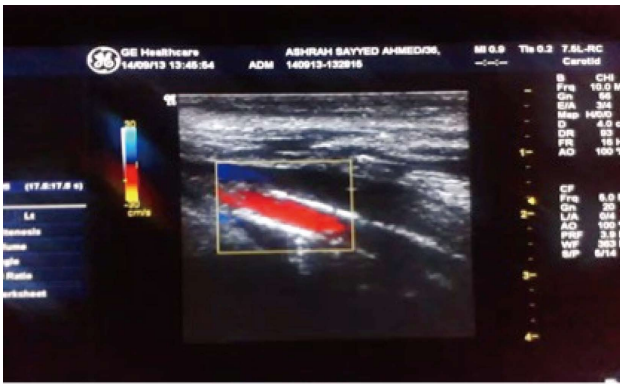

Figure 3 Doppler study of popliteal artery, the vessel wall shows linear calcification.

highest vascular risk and that this information should guide the management[171]. On the other hand, Zoccali *et al*[172] denied VC as a risk factor for ongoing vascular disease. Their opinion relies on many studies, one of them is the recent meta-analysis of different clinical trials on the impact of different phosphate binders on mortality in CKD[173], the ADVANCE trial[174] and the EVOLVE trial[175]. In addition, Wanner[176] criticized any effort offered for diagnosis or treatment of VC as long as all the last mentioned trials failed to change the prognosis in HD patients.

In our opinion, the medical practitioners should do their best effort to prevent this devastating pathology in every CKD patient and not to wait to diagnose its end stage in the dialysis population. This means energetic preventive measures should be offered to every CKD patient all through different stages.

IMAGING OF VC

In 2009, the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines did not recommend the routine screening of VC as long as there is no clear clinical utility[177]. However, in some cases, imaging of VC might help to guide the treatment plan[178]. The gold standard for the quantification of calcification in different vessels is by computed tomography imaging. Plain X-rays can help to identify aortic and peripheral arterial calcifications (Figure 2); Doppler ultrasound is helpful in imaging the carotid, femoral and popliteal arteries and the aorta (Figure 3); echocardiography is valuable tool for visualizing valvular calcification and mammography for breast arterial calcification (BAC). BAC is a useful radiologic sign. It indicates tunica media calcification commonly encountered in CKD[179].

Quantification of VC is achieved using either the Kauppila score, the Adragao scores, the Agatston score, the volume score or the mass score. The Kauppila scores are used to quantify calcification of abdominal aorta, an indicator of intimal calcification[180,181]. The Adragao score is used to quantify VC in the iliac, femoral, radial, and digital arteries. Adragao score reflects medial calcification[182]. The volume and mass scores are quantitative and more reproducible measurements (mm^3 or mg, respectively)[183-185], in addition to being more appropriate for use with modern CT scanners than the Agatston score[186]. However, the Agatston score (semiquantitative) is the most frequently used and reported method in the medical literature so far.

WHEN TO INTERFERE?

Interference for the traditional risk factors must start very early while the patient is still in stage G1. For nontraditional risk factors, we should start in the very early days of stage G2. The severity of arterial stiffness (as an index of atherosclerosis and VC) was found to increase steadily with more advanced CKD from stages 1 to 5[187]. The different factors concerned with VC begin very early when renal damage is still trivial and before hyperphosphatemia ensues. Therefore, the earlier the intervention the better is the impact on morbidity and mortality[188]. The changes in these factors are sequential. FGF23 is the earliest starting during stage G2. Decline of serum calcitriol follows when GFR falls below 60-70 mL/min per 1.73 m^2. PTH elevation follows in the late phase of stage G3a while changes in serum phosphate occur in stage G3b[189]. We would like to emphasize that this sequence of events is triggered by the decreased capability of the injured kidney to manipulate phosphate excretion. FGF23 triggers the increase in the fractional excretion of phosphorus by the surviving nephrons. FGF23 inhibits 1-α hydroxylase enzyme, with subsequent decrease in synthesis of 1α calcidol. Decreased calcitriol synthesis will result in the decline of its serum level, and later in stimulation of parathormone synthesis and secretion. Being the earliest sign of disturbed renal handling of phosphorus, interference should start as soon as FGF23 starts to rise, at a much earlier stage than we do in the time-being[81,190-196].

HOW TO MANAGE?

VC management aims at improvement of survival and

morbidity in the CKD population[197]. However, there is a lack of data to guide management strategies in these patients based on CAC scores[198]. KDOQI guidelines recommend special care of CKD patient if VC is detected in more than one site[199]. VC is not reversible, so far. Accordingly, the successful management is based on how to prevent or to stabilize existent lesions[200].

Management of traditional risk factors among dialysis patients still faces concern about its value. Such factors were found correlating with better survival[201]. Initially, treatment of different traditional risk factors in pre-dialysis CKD patients was based on studies mainly done in cohorts without renal disease, many trials tackling many of such factors in CKD patients have evolved but are still limited[202].

The strict control of blood sugar carries little benefit, if any, to CKD G5D patients with or without diabetes mellitus[203]. However, such control has a positive impact on survival of pre-dialysis diabetic CKD patients. Glycemic control might also delay CKD progression and postpones the need for dialysis[204,205].

Blood pressure control muffles the rate of decline in GFR in pre-dialysis CKD patients[206]. Hypertensive CKD patients should be treated according to KDIGO guidelines[207]. The problem is much debatable when discussing hypertension control in dialysis patients[208]. Home BP carries better prognostic impact when compared to recordings in the dialysis unit. Systolic home BP of 115-145 mmHg is associated with the best prognosis in HD patients[209]. Renin-Angiotensin-system (RAS) blockers stimulate Klotho gene expression in CKD patients. This novel mechanism might clarify the vascular, cardiac and renal protective benefits of such agents[210,211]. The RAS mediated renal damage might be through Klotho gene manipulation[212]. Through their manipulation of Klotho gene, RAS blockers can add a new exciting mechanism for their cardiovascular and renal protective effect.

Aldosterone might induce vascular calcification. We are still waiting for clinical studies to evaluate if there is a protective effect of aldosterone antagonists[213].

CKD patients frequently develop dyslipidemia. Treatment with statins to lower LDL cholesterol is recommended by KDOQI and KDIGO in all adult patients with diabetic CKD and in hypercholesrolemic non- diabetic CKD patient. Such treatment can reduce different cardiovascular events complicating atherosclerosis. However, this treatment does not impact overall mortality in these patients[214,215]. Many trials targeting CKD patients were done using different statins or statin-ezetimibe combination. In CKD G3 patients, pravastatin treatment was associated with significant reduction of coronary events[216]. However, another trial using the same statin failed to show any significant impact on 2^{ry} prevention in patients with early CKD[217]. When statins are used for primary prevention, instead, they reduced the risk of cardiovascular events in stages 1-3 CKD by 41%[218]. On the other hand, all trials comparing statins with placebo in HD patients failed to demonstrate any significant impact on clinical outcome or overall mortality. These trials used atorvastatin, 20 mg daily, in the 4D study, rosuvastatin, 10 mg daily, in the AURORA trial and simvastatin, 20 mg plus ezetimibe 10 mg, in the SHARP study[219-221].

Lifestyle modifications including regular muscle exercise, salt restriction, decrease of calorie intake, and smoking cessation carry significant cardiovascular benefits in the general population. However, we lack data supporting such interventions at all CKD stages[202].

The very early elevation of FGF23 during CKD G2 should stimulate the attending physicians to reduce phosphorus intake in CKD patients starting in the early days of stage 2[222]. Phosphate binders, whether calcium containing or calcium-free, should be avoided in this early stage as long as serum phosphorus level is normal or near normal. The very early use of the phosphate binders might be associated with progression of VC while lowering serum phosphorus and attenuating the progression of secondary hyperparathyroidism[223].

Calcium-based phosphate binders are still very useful to control hyperphosphatemia, but can lead to hypercalcemia and/or positive calcium balance and cardiovascular calcification[224].

Sevelamer hydrochloride and carbonate are resin-based binders that appear to have profiles that would prevent or muffle VC[224]. Treatment of non-diabetic stage 3 CKD patients that have normal serum phosphorus with sevelamer did not lower cardiovascular-related outcomes[225]. These findings reinforce the trend to avoid phosphate binders in early stages of CKD where the serum phosphorus is still normal. On the other hand, when sevelamer was used in hyperphosphatemic stage 3-4 CKD patients, a significant impact on all-cause mortality and the need of dialysis was observed in comparison to calcium carbonate[226]. The main drawback of all calcium-containing phosphate binders is the tendency to increase serum calcium level. The higher the dose ingested the greater the extent of VC[227,228]. Thus their use in cases suffering VC, hypercalcemia, low level of parathormone (PTH) and/or adynamic bone disease has to be restricted[229]. In the US Sevelamer is mainly used in dialysis patients to decrease progression of coronary artery and aortic calcifications[230-235]. On the other hand, the European Medicines Agency recommended its use in hyperphosphatemic patients with CKD not yet on dialysis[236-238]. When incident HD patients were assigned to either calcium-based phosphate binders or sevelamer, and were followed for 44 mo, all-cause mortality was lower in subjects assigned to sevelamer compared to patients assigned to calcium-based binders. However, results were of borderline statistical significance. Another important finding in this study is the significant predictive value of baseline CAC score concerning all-cause mortality[239]. In the "Treat to Goal Study", coronary and aortic calcification progressed in dialysis patients receiving calcium-containing phosphate binders while those receiving sevelamer did not show progression[232]. On the other hand, sevelamer failed to improve mortality rate among prevalent HD

patients when compared to calcium-based binders in the multicenter, randomized trial "the DCOR"[240].

We like to emphasize that while the hyperphosphatemic stage 3-4 CKD patients showed benefits in all-cause mortality[226], and the incident HD showed borderline significantly lower mortality after sevelamer use[239], the same agent failed to show a similar benefit in prevalent HD subjects[240]. We should remember that these different groups are in different stages of evolution as regards VC[9,10,160] and that the baseline score of coronary calcification is a strong predictor of all-cause mortality[239]. This confirms that the earlier the approach the better would be the impact on CKD patient survival.

Sevelamer is not just a calcium-free phosphate binder, but also has additional pleiotropic effects such as correcting certain abnormalities of lipid metabolism[241], significant decrease in inflammatory parameters including IL-6, sCD14 and hs-CRP[242,243], reduction of serum uric acid concentration[244], decrease of serum FGF23[123,245,246], increase of serum level of fetuin-A[236,247] and Klotho[246]. Compared to calcium based phosphate binders, sevelamer improves endothelial function in CKD patients[248]. These results suggest that sevelamer has, beside its hypophosphatemic and calcemic actions, important metabolic, and anti-inflammatory actions that help in decreasing uremic vasculopathy. Sevelamer is more expensive compared to calcium-based phosphate binders[249]. The significant reduction in all-cause mortality and the significantly fewer hospitalizations in the sevelamer group can offset the higher acquisition cost for sevelamer[250].

Lanthanum carbonate (LC) is another non-calcium based phosphate binder. It was reported to improve aortic VC progression[251]. There are no trials studying the effect of LC on either coronary or valve calcification[252]. LC had no impact on over all mortality in CKD patients[251,253]. However, the mortality was significantly lower in patients above 65 years in the LC treatment group compared with calcium based phosphate binders. A similar observation was reported in patients receiving sevelamer in the DCOR study[240,254]. In the only trial looking for the impact of LC on the incidence of cardiovascular events, it failed to show any significant difference compared with calcium-based compounds[251].

Contrary to sevelamer, lanthanum carbonate does not have a consistent effect on FGF23. LC failed to cause reductions in iFGF23 in patients with CKD stage G3-4[255,256]. On the other hand, other studies showed that LC was effective in reducing FGF23 levels in CKD G3[257] and CKD G4 - 5 patients[258]. None of the trials on Lanthanum reported any effect on inflammation or inflammatory biomarkers. Although LC is cheaper and more compliant (Table 1) compared to either sevelamer hydrochloride or sevelamer carbonate[259], our target is not just to control phosphorus level. Sevelamer compounds have got more comprehensive trials that showed significant impact on patient mortality during predialysis stages and in incident HD. No similar trials could be encountered for lanthanum. We are still waiting for such studies to assure non-inferiority of Lanthanum in this field.

The value of nicotinamide (NAM) in phosphate control (as well as its effects on lipid levels) in dialysis patients was explored in some short-term trials[260-262]. However, such trials did not look for either pharmacokinetics or safety. None of these trials studied the impact on VC, FGF23, Klotho or inflammatory mediators.

Iron compounds represent the new class of phosphate binders. Ferric Citrate, Sucroferric oxyhydroxide, and Fermagate (iron-magnesium hydroxycarbonate) were tested in some clinical trials[263]. Most of the clinical studies done so far were using ferric citrate, stressing on phosphate binding and ferrokinetics after short periods of trial. So far, no trials have studied the impact on VC[264-272]. A single study looked for non-inferiority of Sucroferric oxyhydroxide (PA21) compared to sevelamer carbonate concerning phosphate binding[273].

Bixalomer is novel non-calcium, amine-functional polymer that binds phosphate in the gastrointestinal tract and inhibits its absorption. It was approved as hypophosphatemic agent in Japan by June 2012. It proved non-inferiority with much lower adverse effects relative to sevelamer hydrochloride[274].

Salivary phosphorus binding is another approach to reduce serum phosphate level. Chitosan-loaded chewing gum, chewed during fasting periods, may be a valuable add-on to phosphate binders that can lead to a better control of hyperphosphatemia[275].

The possible beneficial effect of bisphosphonates on VC has evolved during the 1970s when their administration was found associated with decreased calcification of soft tissue in animal and clinical trials[276,277]. These observations are probably explained by the paradoxical relation between bone mineral density (BMD) and VC[276-278]. That effect might also be related to the stimulatory action of bisphosphonates on fetuin-matrix Gla protein-mineral complex[279] and their possible inhibitory action on IL-6. Transformation of VSMCs to osteoblasts and calcification of intimal atheromatous lesions might be triggered by IL-6[280]. Bisphosphonates were found to inhibit vascular arterial and cardiac valvular calcifications that develop in rats treated with warfarin[281]. When different members of bisphosphonates were tried in chronic HD patients their anti-calcific effect was favorable in some studies[282-284] and failed in other more recent one[285]. In addition, alendronate failed to withhold the progression of VC in G3-4 CKD patients when compared with placebo for 18 mo[286]. Bisphosphonates are not safe in patients suffering advanced CKD. They can aggravate hyperparathyroidism. They can also lead to adynamic bone disease, osteomalacia or mixed uremic osteodystropy[287]. All the trials of bisphosphonates studied their impact on VC. Only one trial studied the impact of bisphosphonate treatment on cardiovascular outcomes in female CKD patients. This study was retrospective[288].

In the EVOLVE Trial, cinacalcet was tested in chronic HD patients suffering moderate-to-severe 2ry hyperparathyroidism. Inspite of the favorable effects of

Table 1 Different therapeutic interventions used to prevent or withhold vascular calcification progression

CKD stage	Risk factor	Type off interference	Outcome	Ref.
Traditional Risk factors				
G1-G5	Cigarette smoking	Cessation	No evidence	[202]
G1-G5	Overweight	Decrease calorie intake	No evidence	[202]
G1-G5	Sedentary life	Muscle excercise	No evidence	[202]
G1-G5	Diabetes mellitus	Blood sugar control	Improves survival	[204]
			Delays CKD progression	[205]
G1-G5	Systemic hypertension	Blood pressure control	Delays CKD progression	[206]
G1-G5	Dyslipidemia	Statins	Decreased CV morbidity	[221]
CKD Related Risk factors				
G2-G5	↑	Dietary phosphate restriction	↓	[222]
G3b-G4	Hyperphosphatemia	Sevelamer	↓VC, ↓	[226]
G5		Preemptive kidney Tx	↓VC, ↓	[52,295]
Incident G5D	Hyperphosphatemia	Sevelamer	↓VC, borderline↓	[231]
Prevalent G5D	Hyperphosphatemia	Sevelamer or L.C.	↓VC,	[232,240,251]
Prevalent G5D > 65 yr	Hyperphosphatemia	Sevelamer or L.C.	↓VC, ↓	[240,251]

CKD: Chronic kidney disease.

cinacalcet on serum calcium, it failed to decrease the mortality rate or the major cardiovascular events in such patients[175].

We recommend small dose of vitamin D or vitamin D analogues to be given daily as prophylaxis against VC in spite of the lack of clinical trials favoring the use of either native or active vitamin D analogues to prevent VC progression. The rarity of vitamin D toxicity in general and the privileged survival benefits offered by VDRAs administered in small doses even in cases suffering hyperparathyroidism and/or increased calcium and phosphorus levels supports this concept. Some studies reported the association of low vitamin D serum level with extensive VC[289,290]. Vitamin D inhibits renin activity, inflammation, suppresses stimulators of VC and stimulates inhibitors of VC in the uremic milieu[291].

We are still looking for the possible role of vitamin K supplementation in management of VC[292]. Treatment of CKD rats with vitamin K1 suppressed the development of VC[293]. A prospective trial is going on in RDT patients suffering coronary calcification. The effect of vitamin K1 supplementation on the calcification progression in the thoracic aorta and coronary artery will be addressed. All-cause mortality is a secondary end-point. This study may offer an inexpensive agent to treat or prevent VC[294].

Once the patient proceeds to stage 5, pre-emptive kidney transplantation is the best option to improve patient and graft survival in comparison to patients admitted to dialysis or to patients transplanted after starting dialysis[295-298]. In patients starting dialysis, the shorter the dialysis vintage the better is the post-transplant survival[299]. The survival benefit of transplantation compared to dialysis is most probably related to the decreased rate of VC post-transplant compared to the accelerated progress in VC observed in dialysis. To further decrease the rate of calcification progression after transplantation, perioperative vascular imaging and analysis of serum FGF23 might help in appointing patients more likely to have progression of VC Such patients should continue the anti-calcific measures applied to CKD G3 patients. This advice is based on the previous observation of the strong association between baseline CAC score and CAC progression[39,300] and on the recent finding of high serum level FGF23 in KTR even when they have normal graft function[301]. This disturbance of FGF23 appeared to be related to the endothelial cell injury in KTR[302]. Elevated levels of FGF23 may predict increased risks of death and allograft loss[303].

Since the pathogenesis of CUA is not fully elucidated, its treatment is still not uniform[304]. Cinacalcet appeared to reduce the incidence of CUA in HD recipients who have moderate to severe secondary hyperparathyroidism[305]. Sodium thiosulphate[38,304] is used successfully in treatment. Bisphosphonates may be also used[306,307].

CONCLUSION

The new definition and staging for CKD suggested by the NKF-KDOQI in 2002 aimed at stimulation and increased awareness of the medical community to early diagnose CKD[308]. Early diagnosis of CKD gives a great chance to delay the progression of such disease, and to have better chance to deal with the different complications. VC has evolved as the most serious complication in CKD patients endangering their life. The only successful treatment for VC is preventive. This treatment should start as early as the early days of stage G1. Control of blood sugar in diabetic pre-dialysis CKD patients is a mandate. Recommended hemoglobin A(1c) level should be around 7%. Hypertensive CKD patients should be treated according to KDIGO guidelines. Statin treatment should be prescribed according to KDIGO guidelines.

Screening for FGF23 would pick up CKD patients requiring phosphorus handling at much earlier stage when they benefit maximally. However, we are still waiting for epidemiologic studies that would determine normal and target levels of FGF23 and the ideal method of assay.

In these early days, moderation of dietary phosphate intake might suffice. If Serum PTH level is high, we

should measure serum 25-hydroxy vitamin D level[309]. If such level is below 30 ng/mL the patient should be prescribed either vitamin D2 or D3. We are waiting for prospective clinical trials studying the value of recombinant Klotho treatment in normalization of serum FGF23 level and preventing the development or progression of VC. Regular estimation of serum calcium, phosphorus, Ca x p byproduct and PTH should be performed with the frequency recommended by guidelines[310]. Once serum phosphorus starts to rise above normal, strict restriction of dietary phosphorus and prescription of sevelamer should ensue. Other phosphate binders could be used, however, the lack of clear evidence for their effect on Klotho and on cardiovascular morbidity and mortality would postpone their use in the time being till we have strong evidence for these effects. A small dose of vitamin D analogues should be added to all patients passing to stage 3 and beyond. Vitamin K looks promising in preventing or slowing the progression of VC, however, we are still waiting for the results of the ongoing study looking for its efficacy. Once the patient proceeds to stage 5, pre-emptive kidney transplantation is the best option to improve patient and graft survival in comparison to patients admitted to dialysis or to patients transplanted after starting dialysis. In patients starting dialysis, the shorter the dialysis vintage the better is the post-transplant survival. To further decrease the rate of calcification progression after transplantation, perioperative vascular imaging and analysis of serum FGF23 might help in appointing patients more likely to have progression of VC Such patients should continue the anti-calcific measures applied to CKD G3 patients.

In patients maintained on dialysis, non-calcium phosphate binders still carry the privilege of decreased progression of vascular calcification in spite of their failure to impact either cardiovascular morbidity or mortality. HD patients above 65 years of age showed survival benefit after use of sevelamer or LC, the latter is preferred in this age group based on patient compliance and cost of treatment.

Finally we have to emphasize that huge effort is still needed to support many of the above suggestions by well-designed prospective controlled studies to evaluate either efficacy, safety of such interventions beside the precise definition of optimum dosage and frequency of every individual therapeutic modality.

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MINIREVIEWS

Hypertensive disorders in pregnancy

Casey Berry, Mohamed G Atta

Casey Berry, Mohamed G Atta, Department of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, United States

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Correspondence to: Mohamed G Atta, MD, MPH, Department of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine, 1830 E. Monument Street, Suite 416, Baltimore, MD 21287, United States. matta1@jhmi.edu
Telephone: +1-410-9555268
Fax: +1-410-3672258

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Abstract

Renal injury or failure may occur in the context of pregnancy requiring special considerations with regard to fetal and maternal health. The condition of pregnancy itself may be a major factor in such injuries. In addition, for many young women previously known to be healthy, pregnancy may be the first presentation for routine urine and blood testing which may yield previously subclinical renal disease. As such, pregnancy may add complexity to considerations in the management of renal disease presenting coincidentally requiring knowledge of the physiologic changes and potential renal disorders that may be encountered during pregnancy.

Key words: Pregnancy; Hypertension; Preeclampsia; Hemolysis, elevated liver enzymes, and low platelets

Core tip: Kidney disease and particularly complications of hypertensive disorders is one of the dire threats to successful pregnancy. This review highlights advances in our understanding of the pathophysiological processes that drive the development of hypertensive disorders' complications during pregnancy, potential use of biomarkers in predicting these complications, and novel therapeutic approaches under consideration for their great promise in achieving successful pregnancy.

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INTRODUCTION

Pregnancy is a unique arena in the practice of nephrology in which special considerations must be made for a host of factors such as hemodynamics, immunology, metabolism, pharmacology, and embryology. For many young women previously known to be healthy, pregnancy may be the first presentation for routine urine and blood testing which may yield previously subclinical renal disease. Furthermore, renal injury or failure may occur in

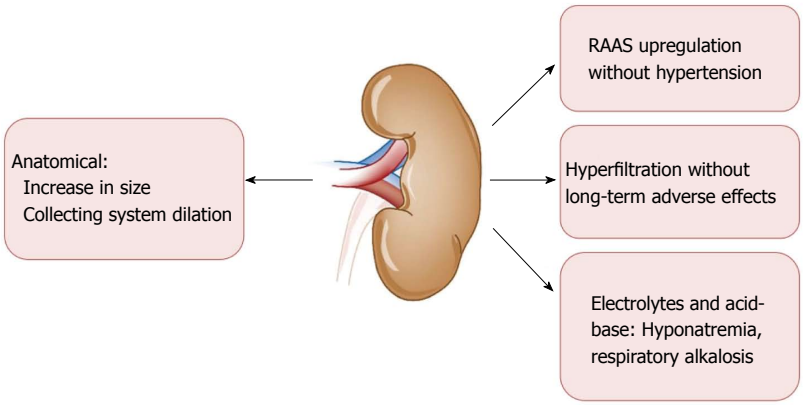

Figure 1 Physiological renal changes during pregnancy.

the context of pregnancy requiring special considerations with regard to fetal and maternal health. The condition of pregnancy itself may be a major factor in such injuries (*e.g.*, as in prerenal injury secondary to hyperemesis gravidarum, or acute cortical necrosis secondary to septic abortion or peripartum hemorrhage) or may simply add complexity to considerations in the management of renal disease presenting coincidentally. The nephrologist may be consulted when patients develop acute kidney injury (AKI) with glomerulonephritic features including refractory hypertension, edema, reduced estimated glomerular filtration rate (eGFR), proteinuria, and occasionally microangiopathy[1], for which preeclampsia is often on the differential diagnosis. This review aims to explore the anticipated physiologic changes in pregnancy before addressing the hypertensive disorders of gestation, of which pre-eclampsia is the most common; affecting approximately 5% of pregnancies worldwide. Our aim is to present a comprehensive overview of the current knowledge on physiologic changes in pregnancy, with special attention paid to the epidemiology, genetics, pathophysiology, diagnosis, and management of pre-eclampsia.

NORMAL ADAPTATIONS EXPECTED IN PREGNANCY

Several unique physiologic changes occur in the context of a normal healthy pregnancy (Figure 1). There is an overall up-regulation of the renin-angiotensin-aldosterone system (RAAS) that begins at the time of the luteal phase of the menstrual cycle and is co-incident with rising estrogen and progesterone levels[2,3]. After fertilization, elaboration of each of these hormones continues in order to support gestation; renin up to eight times, angiotensin up to four times, and aldosterone up to ten to twenty times normal levels[2,3].

Healthy women do not become hypertensive in this context, however, owing either to an estrogen-mediated decrease in vascular responsiveness to these RAAS components, or possibly owing to the counteracting vasodilatory effect of prostacyclins and the ovarian-secreted gestational hormone relaxin[4]. This systemic vasodilation tends to decrease systolic blood pressure by about 10-15 mmHg[5]. This global vasodilation also dilates the renal vasculature resulting in an early and robust increase in glomerular filtration-initially by about 25% and progressing up to 50% by mid-pregnancy. There is an even larger increase in renal plasma flow, around 60%. The result is a state of hyperfiltration that does not actually engender any pathologic injury as in other states of hyperfiltration such as in diabetic kidney disease[6]. Pregnancy is associated with reduction in the single nephron filtration fraction compared to the increase encountered in other hyperfiltration conditions. RAAS up-regulation contributes to sodium and fluid retention, which facilitates plasma volume expansion within the dilated vasculature. Intravascular volume expansion results in a mild dilutional anemia and hyponatremia; serum sodium concentrations may be reduced by approximately 4-5 meq/L[7].

Increased renal volume and length is frequently appreciated on ultrasonography, along with mild non-obstructive hydronephrosis due to uterine compression of the ureters. Pelviectasis is typically more pronounced on the right side, possibly owing to dextrorotation of the uterus and exaggerated by the relative protection of the left ureter provided by the sigmoid colon[8]. This low-grade obstruction may be symptomatic in approximately 30% of pregnant women and can predispose to urinary tract infections.

HYPERTENSIVE DISORDERS OF PREGNANCY

Preexisting and gestational hypertension

An increasing subset of women enter into pregnancy with pre-existing hypertension in the setting of the usual risk factors for essential hypertension such as obesity, race, and advanced maternal age. An estimated 25% of these patients may develop a superimposed preeclampsia syndrome. Hypertensive disorders of pregnancy increase maternal risk of developing AKI in addition to other etiologies as stratified in Table 1. In this relatively young demographic, essential hypertension is less likely to have been present long enough to manifest any clinically apparent end-organ damage so the development of any proteinuria or other renal dysfunction would potentially

Table 1 Selected renal disorders during pregnancy

Renal disease by trimester		
1st trimester	Hyperemesis gravidarum	Worsening of preexisting renal disease
Week 1-12	Cortical necrosis due to septic abortion	↓
	Preeclampsia (> after 20 wk)	↓
	AFLP	↓
2nd trimester	Preeclampsia	↓
Week 13-28	HELLP syndrome	↓
	TTP	↓
3rd trimester	Preeclampsia	↓
Week 29-40	Polyhydramnios	↓
	Extraureteral obstructive hydronephrosis	↓
Post-partum	Post-partum hemolytic uremic syndrome	↓
	Preeclampsia	↓

AFLP: Acute fatty liver of pregnancy; TTP: Thrombotic thrombocytopenic purpura; HELLP: Hemolysis, elevated liver enzymes, and low platelets.

Table 2 Renal disorders and associated maternal and fetal health risks

Maternal and fetal risk by degree of renal impairment		
Stage	**Pregnancy/fetal outcomes**	**Renal/maternal outcomes**
Early CKD Ⅰ-Ⅱ sCr < 1.4 mg/dL eGFR < 70 mL/min Normal BP Minimal proteinuria	Higher risk than general population for preeclampsia, SGA, preterm delivery Counseling: May need specialized care Generally good outcomes	Lower risks for accelerated progression
Moderate CKD Ⅱ-Ⅲ sCr 1.4-2.4 mg/dL eGFR 40-70 mL/min	With more advanced CKD and higher proteinuria: Higher risks of caesarian section, preterm delivery, SGA, and need for NICU	Increased risk of progression during pregnancy and within 6 wk postpartum Counseling: Pregnancy termination doesn't reliably reduce risks for progression
Severe CKD Ⅲ-Ⅳ sCr > 2.4 mg/dL eGFR < 40 mL/min	With more advanced CKD and higher proteinuria: Higher risks of caesarian section, preterm delivery, SGA, and need for NICU care	Increased risk of progression during pregnancy and within 6 wk postpartum
ESRD	Decreased fertility and high fetal mortality except with more intensive hemodialysis Higher risks of preeclampsia, SGA, cervical incompetence, and need for NICU care persist	Increased need for transfusion, worsening hypertension
Post-transplant	± increased risk of fetal loss Increased risk of low birth weight and preterm delivery Significantly increased risk of preeclampsia if hypertensive	Blunted renal physiologic adaptations No anticipated decrease in graft survival but may be associated with decreased maternal life span Increased risk of diabetes, urinary tract infection (due to anatomy, insulin resistance, and immunosuppression)

SGA: Small for gestational age; NICU: Neonatal intensive care unit; CKD: Chronic kidney disease; ESRD: End-stage renal disease.

point to the onset of an overlapping preeclampsia syndrome. In women entering pregnancy with pre-existing renal disease, which may potentially be masked by the effects of hyperfiltration on conventional markers of renal function (*e.g.*, serum creatinine) risks to the mother and fetus can be stratified by the severity of renal insufficiency and modes of renal replacement therapy, if applicable (Table 2)[9].

Clinical features, criteria, and definition of preeclampsia

Preeclampsia is a heterogeneous, multi-system disorder characterized by widespread dysfunction, including glomerular endothelium; it is the most common glomerular disorder in pregnancy. The criteria for diagnosis includes two blood pressure readings at least 4-6 h apart that are greater than 140/90 occurring after 20 wk' gestation in a woman not known to be previously hypertensive[10]. The syndrome may also develop in the 4-6 wk postpartum period[10]. Consequently, preeclampsia is best categorized into early onset/placental (< 34 wk of gestation) *vs* late onset/maternal (> 34 wk of gestation) reflecting that there are potentially different triggering events in pathogenesis as well as the worse maternal-fetal prognoses of early *vs* late preeclampsia. Edema and elevated uric acid levels are also frequently among the constellation of findings but are not strictly part of the definition[10] (Table 3). Either new onset or worsening of pre-existing proteinuria greater than 300 mg in 24 h may be present, but proteinuria itself has been removed from the definition since it is a relatively late marker of

Table 3 Definition of preeclampsia

Updated definition	Supportive clinical signs
2 blood pressure readings	Edema
⩾ 140/90	±
Taken ⩾ 4-6 h apart	Uric acid level ⩾ 7.8 mg/dL
After 20 wk gestation	±
+	Proteinuria (severe ⩾ 5 g/g)
Patient not previously known to be hypertensive	Thrombocytopenia Elevated serum aminotransferase levels Acute kidney injury Pulmonary edema Cerebral/visual disturbances (new onset)

glomerular injury. Higher levels of proteinuria above 5 g/g were once considered to be a marker of severity as well but this has fallen out of favor for the same reason. In the absence of proteinuria, preeclampsia is confirmed when *de novo* hypertension after 20 wk of gestation is associated with maternal or fetal end organ damage which may include thrombocytopenia, elevated serum aminotransferase levels, AKI, pulmonary edema, new onset of cerebral or visual disturbances, or uteroplacental dysfunction. AKI or renal failure can occur, however identifying AKI may be fraught with its own challenges given the lack of a consensus definition of AKI in the pregnant population[10]. Approximately 10%-20% of cases of preeclampsia are severe enough to manifest hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, a thrombotic microangiopathic process (TMA) named for its most notable features of red cell lysis and thrombocytopenia[10]. As such, HELLP is likely a form of atypical HUS that is triggered by pregnancy although transaminitis can occur alone or as part of this syndrome. Adverse cardiovascular and cerebrovascular outcomes may develop if blood pressure is not adequately controlled.

Morphologically, endothelial swelling (Figure 2A) is the cardinal feature on light microscopy which typically resolves approximately eight weeks after delivery, along with the proteinuria and hypertension. However, persistent damage can follow preeclampsia in the form of focal segmental glomerulosclerosis (FSGS) with collapsing features. Loss of glomerular endothelial fenestrae with relative preservation of the podocyte foot processes is expected on electron microscopy along with possible electron-dense deposits in subendothelial and mesangial tissue (Figure 2B). In severe cases or in healing stages one may note increased glomerular cellularity and mesangial interposition. FSGS has been one of the dominant histopathologic lesions in renal biopsies sampled from women with persistent proteinuria following a preeclamptic pregnancy.

Risk factors: Risk of preeclampsia is increased in the setting of maternal endothelial dysfunction. For example, diabetes mellitus can double or quadruple the risk of developing preeclampsia[10,11]. There is also an increased risk for preeclampsia in patients with a first degree relative with preeclampsia[12]. Patients with a history of preeclampsia are known to carry an increased risk of cardiovascular and renal morbidity and there is some evidence that affected women continue to have impaired endothelial-dependent vasorelaxation which may account for this increased risk. However, there are many shared risk factors for preeclampsia and cardiovascular disease such as diabetes, hypertension, obesity, and metabolic syndrome. Another area of growing evidence is the exposure to environmental and innate risk factors that may contribute to increased susceptibility to hypertensive disorders in pregnancy. For example, seasonal variation in preeclampsia and hypertensive disorders of pregnancy have been observed for the better part of a century. Combined findings in a systematic review of 20 preeclampsia studies suggested an increased frequency of episodes during the rainy seasons of tropical climates and the cold seasons of non-tropical climates[13]. Plausible biological mechanisms explaining this association include higher blood pressures as well as wider daily blood pressure and body temperature variations during these seasons, reduced physical activity and dietary changes, decreased vitamin D levels, and increased infections. Along the same line and not surprisingly, plant-based diets higher in fiber and potassium, cereals, dark bread, and low-fat dairy may be associated with reduced preeclampsia risk[14]. Similarly, vegetable-laden low protein diets (not more than 0.6-0.7 g/kg per day) seem to confer beneficial effects on clinical variables of renal health during pregnancies in cohorts of women with chronic kidney disease (CKD)[15].

Genetics and paternity: While much has been published about the maternal and placental pathophysiologic roles in preeclampsia, a growing area of research is contributing to knowledge about the paternal role as well, as summarized in a recent review by Katsi *et al*[16]. Among the early implications for a paternal role are the early observations that a family history of preeclampsia places one at increased risk for the syndrome and that multiparity (if prior pregnancies were uncomplicated) decreases one's risk of preeclampsia, unless there is a change in paternity. This latter fact has been understood to be due to maternal mucosal immune tolerance mechanisms as mediated by human leukocyte antigens. Supporting this hypothesis are reports of relatively increased risks of preeclampsia in women using barrier contraceptive methods, couples with shorter durations of sexual relationships prior to conception, and in women undergoing fertility assistive therapies who receive oocytes fertilized with surgically obtained sperm rather than their partner's ejaculated sperm. Other observations suggest that immune tolerance is not likely to be the only key in understanding the paternal role. Interestingly, men who father one preeclamptic pregnancy may be more likely to father a preeclamptic pregnancy with a

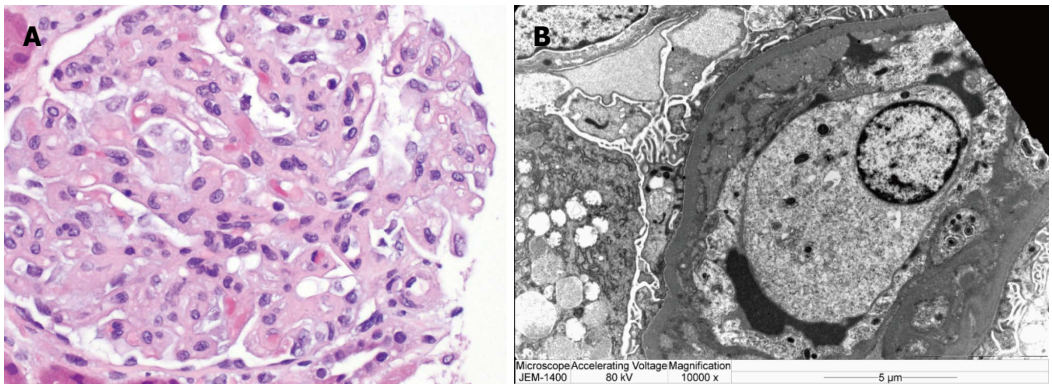

Figure 2 Light (A) and electron (B) microscopy. A: Glomeruli with capillary loops occluded by swollen endothelial cells, *e.g.*, endotheliosis; B: Capillary loop with subendothelial widening by flocculent material and occlusion by swollen endothelial cells. Courtesy of Naima Carter-Monroe.

different woman. Also, in a bimodal pattern, paternal age > 45 or < 25 appears to be another risk factor for preeclamptic pregnancy. Paternal race may factor into risk of preeclampsia, such as when differing from maternal ethnicity; and there appear to be decreased rates of preeclampsia in cases of Asian paternal ethnicity.

Pathophysiology: "Disease of theories": Numerous mechanisms have been proposed regarding the etiologies of preeclampsia, which has earned it the nickname "the disease of theories[12]". Mapping these different mechanisms may help one day to elucidate the different phenotypes and their respective mechanisms. Preeclampsia is a very heterogeneous disease, and it may be better thought of as an umbrella term for multiple different subtypes driven by a variety of different processes to varying extents which have yet to be fully elucidated. The predominant paradigm at present is that there is a fundamental imbalance between proangiogenic drivers and antiangiogenesis. Research in recent years has yielded significant advances in identifying early biomarkers, especially within the proposed pro- and anti-angiogenic pathways. The pathogenesis involves a two stage process wherein in the first stage uterine spiral arteries are incompletely remodeled leading to vasospasm and placental ischemia and in the second stage ischemic placental tissue releases systemic mediators or angiogenesis and inflammation in the the maternal circulation with resulting downstream endothelial injury.

Fetal endovascular cytotrophoblastic cells implant in the uterine endometrium and myometrium during the phase of embryologic development known as placentation, occurring between 8 and 18 wk of gestation. Cytotrophoblasts subsequently invade and remodel uterine spiral arteries to augment blood flow to meet the oxygen and nutrient demands of fetal growth. Remodeling reduces smooth muscle cells in the infiltrated vascular segments, facilitating dilatation and a resulting reduction in ureteroplacental pressure and velocity of flow. In the placentas of preeclamptic women-for reasons that are not entirely clear - this trophoblastic invasion fails to transpire adequately and the result is tortuous, thick-walled, incompletely remodeled vessels prone to spasms among retained vasoactive smooth muscle. The consequence is placental hypoxia, oxidative stress, and subsequent intermittent fetal hypoperfusion. The placental response is expression and secretion of anti-angiogenic and pro-inflammatory factors which may induce maternal hypertension and proteinuria[18].

Angiogenic imbalance: For more than a decade the preeclampsia syndrome has been understood to be associated with elevated circulating levels the molecule "sFlt-1"; the placental synthesis and release of which is believed to be triggered by the placental ischemic and reperfusion injury alluded to above. Abbreviated for "soluble fms-like tyrosine kinase", sFlt-1 is a circulating receptor for vascular endothelial growth factor (VEGF), on which it has an antagonistic effect. In the healthy kidney, VEGF is produced by epithelial podocytes to maintain slit diaphragm integrity and to regulate the health of endothelial cells that express receptors to VEGF. Circulating proangiogenic molecules such as VEGF and placental growth factor (PlGF) are scavenged by sFlt-1 (the soluble receptor to VEGF) and as a result perturbs the balance between pro- and antiangiogenesis, skewing this balance towards the latter. Binding sFlt-1 to proangiogenic regulators prevents these vasodilatory effectors from interacting with their receptors on the endothelial cell surface, impairing nitric oxide-mediated vasodilation. The result is widespread endothelial dysfunction with multiorgan implications including notably the renal manifestations of preeclampsia such as hypertension and proteinuric glomerular dysfunction.

Automated assays for the detection and measurement of circulating of sFlt-1 and PlGF levels have been developed but are not yet in widespread use[19]. In a clinical setting the ratio of these markers may perhaps soon provide indices of overall antiangiogenic activity, offering a potential early prediction tool for the development of preeclampsia well before the clinical onset of disease. Another diagnostic dilemma is the later gestational exacerbations of preexisting hypertension

and proteinuria which can be difficult to interpret and presents a diagnostic challenge in the identification of superimposed preeclampsia in this population. Bramham *et al*[20] recently published their evaluation of the performance of PlGF concentrations as predictors of superimposed preeclampsia (with a clinical endpoint of requiring delivery within 14 d) in patients with CKD and/or chronic hypertension[20]. In this study, PlGF levels less than the fifth centile performed well in the detection of superimposed preeclampsia in pregnant women with CKD and/or chronic hypertension, with specificity and negative predictive values greater than 80%. sFlt-1, B-type natriuretic peptide, neutrophil gelatinase-associated lipocalin (NGAL), and relaxin were also evaluated but were concluded to have less promising diagnostic discriminatory potential[20].

Soluble endoglin (sEng) is another known detectable antiangiogenic molecule that behaves as an inhibitory receptor for the cytokine TGF-β1[21]. Similar to sFlt-1, it can be found to be at notably increased serum levels as early as 2 to 3 mo prior the clinical onset of preeclampsia. It may offer improved predictive accuracy when used in combination with the ratio of sFlt-1 to PlGF. In normal pregnancies, the sFlt-1 to PlGF ratio is an s-shaped curve with a steep rise in the first 5-10 wk followed by a stagnation and a subsequent third trimester rise that progresses until labor and delivery.

In a nested case control study serum levels of total sFlt-1, free PlGF, and free VEGF in 120 women with preeclampsia were tracked throughout pregnancy and matched to normotensive controls by gestational age[22]. In the preeclamptic group mean sFlt-1 levels were noted to rise in late gestation; this group also consistently had lower PlGF levels (demonstrating the high sFlt-1 to PlGF ratio associated with an antiantiogenic milieu). Circulating levels of sFlt-1 were noted to increase on average five weeks before the clinical onset of preeclampsia and the degree of rise correlated with disease severity.

Oncology patients treated with drugs targeting VEGF can develop a "preeclampsia-like syndrome" with severe hypertension, proteinuria, and edema[23]. Anti-VEGF drugs have been an important mainstay in cancer therapy owing to their anti-angiogenic effects, exerted through a variety of pharmacologic mechanisms[23]. Examples include anti-VEGF monoclonal antibodies (*e.g.*, bevacizumab), decoy receptors (*i.e.*, VEGF-Trap drugs), and multi-target tyrosine kinase inhibitors (MTKIs), which interfere with the VEGF signaling pathway (*e.g.*, sorafenib, sunitinib and brivanib)[23]. These drugs have been used successfully in renal and gastrointestinal malignancies[23]. Hypertension is an important side effect, along with GI and skin toxicities. Renal side effects that have been reported include proteinuria and acute renal failure, specifically with bevacizumab and sunitinib[23]. In some cases, there have been associated renal failure and biopsy-proven TMA along with endotheliosis and effacement of foot processes[23]. These side effects seem to occur in a dose-dependent manner and have been observed to resolve with treatment cessation[23].

Podocyte shedding: Another feature noted on kidney biopsy specimens in patients on these anti-VEGF therapies is a down-regulation in the expression of podocyte slit diaphragm proteins such as synaptopodin, nephrin, and podocin[24]. This helps to strengthen the link between the upstream antiangiogenic environment in preeclampsia and the downstream glomerular injury and proteinuria[24]. Although proteinuria is no longer considered essential for the diagnosis of preeclampsia, it remains a hallmark of the disorder, differentiating it from other hypertensive disorders of pregnancy as well as from other proteinuric diseases that may be co-incident with pregnancy. Urine sediment is typically "bland" in preeclampsia-without cells or casts-but there may be detectable podocytes and podocyte specific proteins[25]. There has been a recent diagnostic focus on detecting these sloughed podocytes in the urine of preeclamptic women even before proteinuria develops[25]. The degree of podocyturia correlates positively with that of proteinuria; and podocyte damage and shedding may affect renal function for years following a pregnancy complicated by preeclampsia[25,26]. This may someday serve as another methodology for early detection, however currently available lab techniques need more development[25]. Podocytes can be cultured from urine samples, although not quickly enough to be of clinical utility[25]. Cytospin techniques for detection may be automated, however there is a loss of sensitivity and specificity owing to contamination with other cellular debris[25]. Polymerase chain reaction (PCR) and mass spectrometry are anticipated to provide the most sensitive and specific detection methods but are not yet clinically available[25].

Anti-angiotensin Ⅱ type 1 receptors: Agonistic antibodies to angiotensin Ⅱ type 1 receptors (AT1-AA) were first described in 1999[27]. These are immunoglobulins of the IgG3 subclass which, by agonizing AT1 receptors, lead to enhanced sensitivity to angiotensin II thus influencing increased sodium retention and vasoconstriction. It remains unclear whether these antibodies are the cause or effect; however agonistic antibodies to AT1-AA may be an upstream trigger of increased sFlt-1 expression[28].

Vasodilatory gases and heme oxygenase pathway: Other vasodilatory gases (in addition to nitric oxide discussed above) may offer mechanistic insight and therapeutic value[29]. There exists growing evidence that the enzyme heme oxygenase and its byproduct carbon monoxide may play a protective role in preeclampsia[29]. Heme oxygenase converts heme to bilirubin and biliverdin; both of which are potent antioxidants[29]. Carbon monoxide (CO) is released in this process and is thought to be an important mediator in maintaining placental vasodilation and healthy development[29]. Supporting this are studies demonstrating reduced end-tidal CO levels in women with preeclampsia (perhaps demonstrating decreased heme oxygenase activity); further, women who smoke and live in areas with higher ambient CO appear to have

less epidemiologic risk of preeclampsia[12,29].

Asymmetric dimethylarginine (ADMA) is an endogenous molecule known to competitively inhibit the activity of nitric oxide synthase. Its metabolism is closely associated with homocysteine (Hcy); which, along with ADMA can be found at elevated concentrations in disease patterns characterized by endothelial injury[30]. Hcy is an upstream effector of oxidative stress associated with ischemic injury and CKD[31] and can be found at increased levels in both obesity and vitamin B deficiencies[30]. López-Alarcón *et al*[30] recruited 411 women from two obstetric hospitals in Mexico focused on high risk pregnancies (excluding smokers, diabetics, and women with hypertension) to monitor monthly serum levels of these potential biomarkers. Approximately 20% of the follow-up group went on to develop preeclampsia and tended to have higher Hcy and ADMA concentrations at baseline despite having values within the normal ranges reported for healthy pregnant women. Though there were no detectable differences between groups with varying degrees of preeclampsia severity, serum levels gradually increased throughout pregnancy in the preeclampsia group compared to women who did not develop pregnancy complications (even after adjusting for obesity and nutritional status), allowing authors to postulate that the detection of increases in serum concentrations of these molecules may allow for early prediction of preeclampsia risk[30].

Placental protein-13: Placental protein 13 (PP-13), first discovered in 1983 by Dr Hans Bohn, is produced by the syncytiotrophoblastic layer in early placental implantation and remodeling, and is thought to be shed in the setting of ischemic placental stress and inflammation[18]. It has been evaluated in an ever-growing body of literature with regards to its capacity to be used as a clinical marker of placental pathology. Second and third trimester levels of PP-13 have been shown to rise in preeclampsia compared to normal pregnancy in a manner correlated with severity[18]; however, conflicting reports exist regarding whether detectable levels can be associated in a predictable way with preeclampsia and may vary demographically when accounting for age, ethnicity, and maternal ABO blood type[32]. According to Seravalli *et al*[32], however, first trimester levels of PP-13 are not likely to independently identify increased risk of preeclampsia in a population at low risk for placental dysfunction although in their cohort of 908 women at low risk for preeclampsia, lower levels of first trimester PP-13 were identified in women with higher BMI, perhaps reflective of metabolic syndrome which is thought to be a risk factor for adverse pregnancy outcomes[33]. Confusing this significance somewhat is the finding that cigarette smoking was associated with a profound decrease in first trimester PP-13 levels; cigarette use has been an environmental exposure consistently associated with a reduced risk of preeclampsia despite other negative placental effects such as fetal growth restriction[34,35].

Urine congophilia: Recently reported findings by McCarthy *et al*[36] appear to confirm the presence of increased levels of amyloid protein in the urine of women with preeclampsia, CKD, and CKD with superimposed preeclampsia compared to healthy pregnant women and women with chronic and gestational hypertension. "Congophilia" is a term used to describe the retention of Congo red dye in a specimen which indicates the presence of amyloid, an aggregate of inappropriately folded proteins thought to be generated by stressed endoplasmic reticulum in the ischemic placenta. Also noted was a significant positive correlation between the magnitude of congophilia and urine protein to Creatinine ratios. Further research is needed to elucidate how this method may be utilized clinically to distinguish between renal impairment, early and late term preeclampsia, and other pathologic processes that activate the unfolded protein response pathway in endoplasmic reticulum[36].

CLINICAL MANAGEMENT OF PREECLAMPSIA

Preeclampsia is primarily a placenta-driven disease process; thus delivery is the only definitive treatment. Indeed, levels of key mediators such as sFlt-1 have been noted to fall within 48 h post-partum. The desirability and safety of delivery may depend on clinical considerations such as fetal gestational age, signs of fetal or maternal distress, or severity such as progression to eclampsia as indicated by the presence of seizures[10].

Pharmacologic

Though it does little to reverse or correct the placental under-perfusion that is thought to be driving preeclampsia, aggressive blood pressure control is another essential mainstay in preeclampsia management[11]. The primary goals are to prolong gestation in order to allow further fetal growth and development and to prevent maternal cerebro- and cardiovascular catastrophes[11]. Angiotensin-converting enzyme (ACE) inhibitors and angiotension receptor blockade (ARB) therapies are very effective but contraindicated during any trimester of pregnancy[37]. Their use is associated with "fetal renin-angiotensin system blockade syndrome" characterized by impaired tubular development and oligohydramnios, among multiple devastating other effects[37]. Angiotensin Ⅱ is essential in the regulation of umbilical-placental blood flow and maintenance of GFR in the low-pressure circulation of the fetus[37]. Drugs that inhibit renin directly, such as the drug "aliskiren" are expected to have similar effects to ACE inhibitors and ARBs[37]. There are no case reports of fetal exposure to these drugs, but at present they should be avoided[37]. Labetalol, hydralazine, methyldopa, and nifedipine are the antihypertensives that have the best safety profile and are the typical go-to agents in

pregnancy[11,38].

Magnesium: Intravenous magnesium sulfate ($MgSO_4$) is the treatment of choice for prevention and treatment of recurrent seizures. Infusions are often started 48-72 h prior to delivery induction once preeclampsia is suspected or diagnosed. This allows time for fetal lung maturation after dosing corticosteroids, typically given concomitantly. Dosing may be approached more empirically in patients with normal renal function, however close serum (at least every 6 h) and clinical monitoring of magnesium levels is advised in renal insufficiency to avoid toxicity. While neuroprotective at therapeutic levels, $MgSO_4$ levels above 4.8 mg/dL can lead to central and respiratory depression or cardiac arrest. Calcium gluconate is the appropriate antidote[39]. $MgSO_4$ also has synergistic blood pressure lowering activity with nifedipine[40] and may have anti-inflammatory effects as well *via* AT1-AA which has yet to be further elucidated[17].

Aspirin: Aspirin administration to reduce preeclampsia risk has been an important research question since the 1970s, with more than 50 published trials and several recent meta-analyses[41]. It has been hypothesized that aspirin facilitates trophoblastic invasion of the uterine spiral arteries[41]. Some of its benefit may be due to the inhibition of synthesis of platelet thromboxane, a potent vasoconstrictor produced by endothelial cells. However, data to support this strategy has been conflicting[41]. Since there may be up to 50% risk reduction and there is little harm other than the usual contraindications to aspirin, guidelines currently recommend initiating aspirin in the highest risk patients (such as women with pre-existing diabetes)[11] early on, ideally in the first trimester[41,42].

Statin: Statin use has also been under evaluation-plausibility lies with their known anti-inflammatory properties as well as their demonstrated ability in mouse and *in vitro* studies to inhibit cytokine-mediated release of sFlt-1[43,44]. Statins are also thought to have a positive influence on endothelial health by increasing the bioavailability of nitric oxide, PlGF, and VEGF[44]. Pravastatin has emerged as the only possible safe agent from this class, due to its inability to cross fetal membranes into the embryonic compartments[43]. Simvastatin, lovastatin, and atorvastatin are all lipophilic and able to equilibrate between maternal and fetal compartments where these agents may interfere with cholesterol-mediated cell signaling and result in fetal central nervous system, renal, and limb defects[43].

Metformin: Metformin is known to be safe in pregnancy and is currently used to treat gestational diabetes mellitus[45]. It is hypothesized to reduce sFlt-1 and sEng secretion by way of its effect on reduction of mitochondrial electron transport chain activity and downstream inhibition of hypoxic inducible factor 1α[45]. Recently, *in vitro* and *ex vivo* experimentation demonstrated reduced sFlt-1 secretion from metformin-treated endothelial and placental cells in a dose-dependent manner[45]. Clinical trials assessing the effect of metformin on primary outcomes such as hypertension and preeclampsia have yet to be published[45].

Calcium: As of 2013, the World Health Organization recommends 1.5-2.0 g of elemental calcium daily in three divided doses from 20 wk' gestation until term in all pregnant women in areas where calcium intake is low, particularly those at higher risk of gestational hypertension[46]. Evidence to support calcium supplementation to prevent preeclampsia has been conflicting and remains controversial. In 1996, Bucher *et al*[47] evaluated 14 randomized controlled trials involving 2459 women and found benefits in blood pressure and preeclampsia incidence reduction supporting the use. The following year, however, the NIH study Calcium for Preeclampsia Prevention (CPEP) concluded from a randomized controlled clinical trial of twice as many healthy nulliparous women that calcium supplementation did not reduce blood pressure, adverse perinatal outcomes, or the incidence or of preeclampsia, nor did it delay onset[48]. In the decade following, subsequent large-scale meta-analyses have supported the practice, particularly in developing countries where dietary calcium intake may be relatively lacking, as well as in otherwise healthy high-risk populations[49,50]. Calcium supplementation in this context has not been shown to increase risk of adverse effects such as nephrolithiasis[48].

Heparin: Heparin has been explored as a possible way to augment the excretion of sFlt-1. A recent systematic review and meta-analysis evaluated six randomized, controlled trials and concluded that the use of low molecular-weight heparin (LMWH) resulted in risk reductions in women who had any previous history of placenta-mediated pregnancy complications[51]. The mechanism of the potential benefit of LMWH is not yet well understood, but the LMWH molecule is thought to mobilize sFtl-1 into circulation from heparan-bound sites in extracellular matrix[51]. Heparan is a polysaccharide structurally similar to heparin which is known to sequester and regulate the release of VEGF and other cytokines involved in neovascularization[51,52].

Extracorporeal

Potential therapeutic solutions may lie within restoration of angiogenic balance, for example *via* the antagonism of sFlt-1 and subsequent blockade of its pathologic effects. One strategy involves infusion of sFlt-1's natural ligands VEGF and PlGF at doses high enough to provide systemic saturation. Attempts have been made in animal trials to induce adenoviral synthesis of VEGF as well as by direct infusion of VEGF in mice[53,54]. Alternative potential therapeutic strategies may include the administration of anti-sFlt-1 antibodies or small molecules that reduce sFlt-1 production (such as small interfering "siRNA")[55].

Given the potential adverse effects of novel agents

introduced into maternal circulation and unknowns regarding the ability of such molecules to traverse the placenta, early experiments have instead attempted to remove circulating sFlt-1 with an extracorporeal device. A recent open pilot study was conducted to evaluate the safety and potential efficacy of therapeutic apheresis with a plasma-specific dextran sulfate column to remove circulating sFlt-1 in 11 pregnant women with very preterm preeclampsia[55]. At physiologic pH, sFlt-1 circulates in blood with a strongly positive charge. The dextran columns used are negatively charged, are approved for safe use in pregnancy, and have already been used in therapeutic apheresis for familial hyperlipidemia. Circulating sFlt-1 can be removed selectively, leaving placental sFlt-1 *in vivo*, which may be essential for placental health maintenance. In the treated group, the average sFlt-1 reduction was 18% and the average proteinuria was decreased by an average of 44%[55]. Pregnancy continued for eight days in women treated once and 15 d in women treated multiple times. There were no observed adverse effects or infant deaths. Both groups demonstrated similar short-term neonatal outcomes; neonates in the treatment group required fewer days on supplemental oxygen. Without a controlled trial, it remains unknown whether or not some of the therapeutic benefit derives from the removal of other unmeasured factors by the dextran columns, such as LDL and fibrinogen. Studies using ligand-specific apheresis columns (*e.g.*, configured with anti-sFlt-1 Ab or VEGF) would be informative in determining the relative contribution of sFlt-1 depletion *vs* depletion of other potential mediators[55].

CONCLUSION

Pregnancy is marked by several key physiologic RAAS driven changes that should not result in hypertensive pathology in normal gestation, yet hypertensive disorders in pregnancy abound. Preeclampsia is the most common among these; it is an exceptionally heterogeneous disease that contributes to at least three million pre-term births each year. Placental dysfunction is the fundamental etiology of preeclampsia and mediates the features of the syndrome *via* the systemic release of angiogenic molecules, typically late in pregnancy and signified by the principal clinical findings of hypertension and proteinuria[56]. Angiogenic imbalance seems to be at the root of this disorder, resulting in maternofetal endothelial pathology and renal end-organ damage with an almost glomerulonephritic or nephrotic phenotype[1]. In recent years, knowledge regarding the pathophysiology of preeclampsia has increased markedly. Understanding of this disease process has been significantly advanced by the discovery of the factor sFlt-1 and its placental source, antiangiogenic behavior, and role in diminished glomerular endothelial health and likely holds the key to future advances in prognostication, diagnosis, and treatment of a condition associated with a significant amount of cardiovascular and renal morbidity[56]. To date, preventative measures and screening tools are relatively lacking, treatments are directed at the management of overt clinical manifestations, and delivery remains the only definitive cure; thus, a strong need persists for the expansion of detection and treatment options for this disease which has seen few therapeutic advances in recent decades.

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MINIREVIEWS

Carbon dioxide: Global warning for nephrologists

Marco Marano, Anna D'Amato, Alessandra Cantone

Marco Marano, Anna D'Amato, Hemodialysis Unit, Maria Rosaria Clinic, Pompeii, 80045 Naples, Italy

Alessandra Cantone, Bayview Physicians Group, Suffolk, VA 23462, United States

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Correspondence to: Marco Marano, MD, Hemodialysis Unit, Maria Rosaria Clinic, via Colle San Bartolomeo, 50, Pompeii, 80045 Naples, Italy. marano965@gmail.com
Telephone: +39-081-5359517
Fax: +39-081-8502821

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Abstract

The large prevalence of respiratory acid-base disorders overlapping metabolic acidosis in hemodialysis population should prompt nephrologists to deal with the partial pressure of carbon dioxide (pCO_2) complying with the reduced bicarbonate concentration. What the most suitable formula to compute pCO_2 is reviewed. Then, the neglected issue of CO_2 content in the dialysis fluid is under the spotlight. In fact, a considerable amount of CO_2 comes to patients' bloodstream every hemodialysis treatment and "acidosis by dialysate" may occur if lungs do not properly clear away this burden of CO_2. Moreover, vascular access recirculation may be easy diagnosed by detecting CO_2 in the arterial line of extracorporeal circuit if CO_2-enriched blood from the filter reenters arterial needle.

Key words: Acid-base assessment; Bicarbonate; Carbon dioxide; Hemodialysis; Metabolic acidosis; Mixed disorders; Ventilatory response; Expected pressure of carbon dioxide; Vascular access recirculation

Core tip: Partial pressure of carbon dioxide (pCO_2) should be always taken into account for comprehensive assessment of acid-base imbalances of hemodialysis patients, also because respiratory disorders are very common in this population. To infer a respiratory disorder superimposing to metabolic acidosis, nephrologists should compute the expected pCO_2 complying with the reduced bicarbonate concentration. Moreover, they have to take in account CO_2 load from dialysis solution, because this burden may be harmful if ventilatory compensation does not properly occur. Finally, checking an increase of pCO_2 in arterial line of extracorporeal circuit is an easy and reliable method to discover vascular access recirculation.

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INTRODUCTION

There is widespread awareness about carbon dioxide's (CO_2) effects on global warming of the Earth. A similar recognition would be desirable about the key role of CO_2 in nephrology, but this topic is actually under-recognized. This review aims to issue a global warning about CO_2 in the field of renal replacement therapies.

To date, nephrologists have always focused on serum bicarbonate (HCO_3) concentration and the latter, as marker of metabolic acidosis, has been associated with mortality risk in hemodialysis patients. The finding of a low HCO_3 value has been always regarded as a sign of metabolic acidosis, but respiratory alkalosis also is featured by decreased HCO_3 concentration. Hence, diagnosing metabolic acidosis based on the latter parameter clearly neglects serum HCO_3 modifications that are secondary to respiratory disorders. However, as this "respiratory bias" on serum HCO_3 has been recently highlighted, from now on, a comprehensive assessment of acid-base parameters should be taken into account; it is mandatory, therefore, to include partial pressure of CO_2 (pCO_2) as an important parameter to characterize acid-base imbalances and estimate mortality risk in hemodialysis population. In these patients, respiratory acid-base disorders have been recently found in a large percentage and this should further prompt nephrologists to deal with the pCO_2 complying with the reduced HCO_3 concentration. Mixed disorders occur if measured pCO_2 is not consistent with the expected value.

Next point that will be discussed in this review is the forgotten issue of CO_2 load from dialysis solution. Dialysis solution needs to be acidic to avoid salt precipitations; at the same time, it has to increase patient's blood pH. CO_2 allows to meet both goals, if patients' lungs function is not impaired. In fact the considerable amount of CO_2 in the final diluted dialysis fluid keeps the pH low, preventing salt precipitation. Then, this volatile acid easily and quickly reaches patient's bloodstream and it is cleared by lung ventilation as well. As a result of CO_2 clearance and of HCO_3 addition from dialysis solution, patient's blood pH increases. When this clearance does not happen properly, "acidosis by dialysate" may occur. This syndrome is characterized by early hypercapnia followed by typical, *i.e.,* hypoxic, respiratory failure.

Finally, we will point out that the large amount of CO_2 moving from dialysis solution to the extracorporeal circuit may allow to detect vascular access recirculation if blood returning from the filter reenters arterial needle. Basics of "RecirCO_2lation test" based upon detecting CO_2 in the arterial line of extracorporeal circuit will be outlined.

ACID-BASE STATUS OF HEMODIALYSIS PATIENTS

Bicarbonate and beyond

Since a slightly decreased pre-dialysis HCO_3 concentration has been proven to lead to lower risk of death in hemodialysis patients[1], many efforts have been made to better characterize such risk. Results from Dialysis Outcomes and Practice Patterns (DOPPS) study[2] depicted such relationship as a U-shape curve (Figure 1A): Either very low and very high serum HCO_3 concentrations were associated with higher risk of death. The authors of this landmark study concluded that moderate predialysis acidosis seems to be associated with lower relative mortality risk than what observed in patients with normal ranges of midweek predialysis serum HCO_3 concentration or severe acidosis[2].

In fact the acid-base status of hemodialysis patients was inferred by serum HCO_3, alone; neither pH or pCO_2 were taken into account, because they were unavailable. Furthermore, true serum HCO_3 concentration had not even been measured as the authors dealt with total CO_2 content, however the latter amount is only slightly changed by large fluctuations of partial pressure of CO_2 so that this parameter may be properly used as tantamount to serum HCO_3 concentration. Conversely, it should be noted that serum HCO_3 concentration changes are not exclusively due to metabolic disorders and that this assumption may be misleading because completely neglects the effects of respiratory acid-base disorders on HCO_3 value. These disorders have never been taken into account, but likely exist because DOPPS population was characterized by a burden of comorbidity conditions, including heart and lung diseases known to be associated both with respiratory acidosis and alkalosis.

Another large population study[3] is based on the same assumption. This study confirmed the high risk of death associated with low HCO_3 concentration, however if it was higher than the reference range risk did not increase (Figure 1B). Again acid-base status was inferred by the HCO_3 value alone, but to answer the question whether it is better for an hemodialysis patient to be acidotic or alkalotic - that authors asked - a complete assessment of acid-base parameters is mandatory. Similar findings (Figure 1C) were later reported by Tentori *et al*[4] also in DOPPS cohort, again lacking complete acid-base assessment.

More recently, Yamamoto *et al*[5] failed to find any relationship between serum HCO_3 concentration and mortality risk in a Japanese hemodialysis population (Figure 1D), but remarkably they found a strong association between pre-dialysis pH and mortality risk. Moreover, and above all, they provided all acid-base parameters and, in turn, allowed us to have for the first time the picture of acid-base disorders in a large hemodialysis population. As largely expected, the mean pH value was close to the lower limit of normal reference range, mean HCO_3 concentration was 20.5 mEq/L and pCO_2 was slightly under its normal value[5]. At a first glance, it would seem to be nothing else but mild metabolic acidosis with normal ventilatory response, but looking deeper into their data an unexpected presence of respiratory disorders may

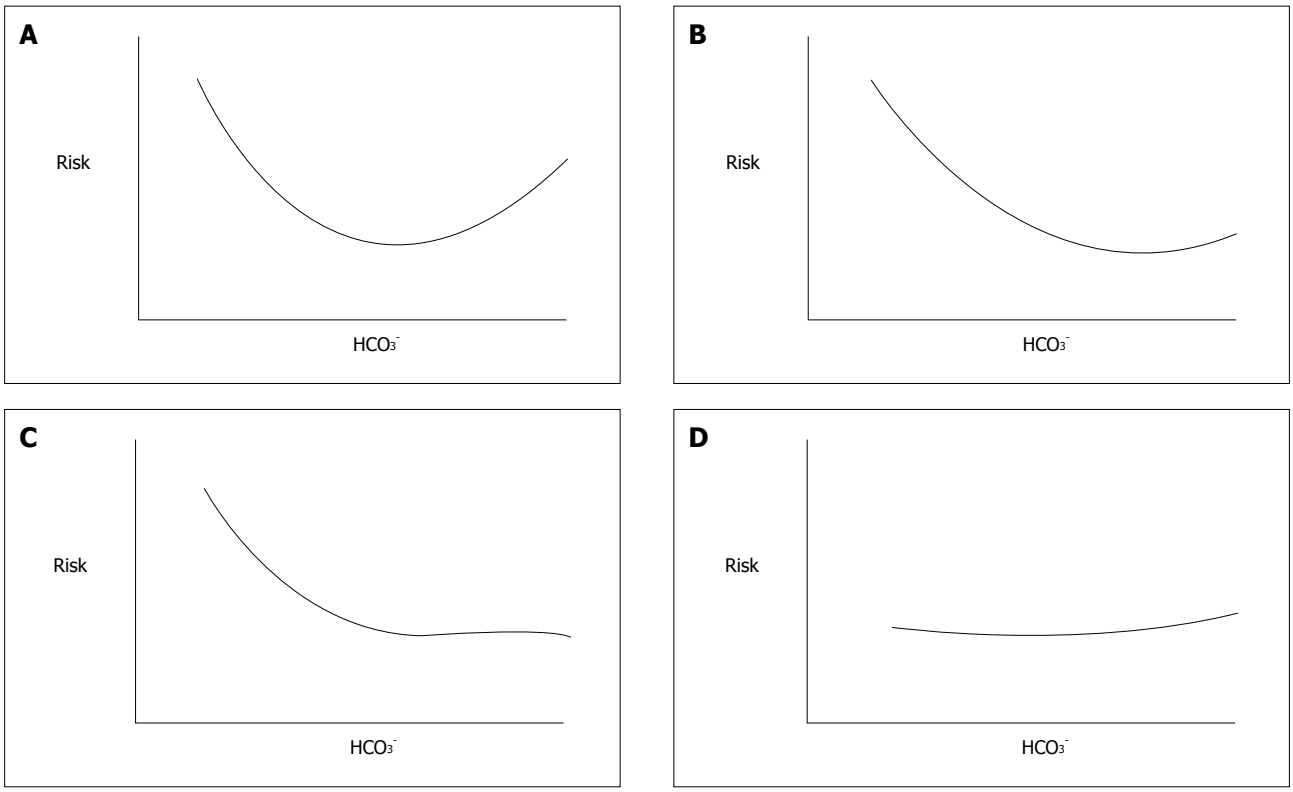

Figure 1 Risk of death and serum bicarbonate concentration in hemodialysis patients. Trend of risk inferred by data from Bommer *et al*[2] (A), Wu *et al*[3] (B), Tentori *et al*[4] (C), Yamamoto *et al*[5] (D). HCO_3: Bicarbonate concentration.

be predicted. In fact, patients in the lowest quartile of pH have the lowest mean value of HCO_3 concentration but higher pCO_2 value than patients in the highest pH quartile. This conflicting pattern of pCO_2 with respect to that of HCO_3 can be exclusively due to a superimposing respiratory acidosis in the lowest pH quartile group. Moreover, in the highest pH quartile group (*i.e.*, pH $\geqslant$ 7.40) HCO_3 concentration was lower than, not higher than, the normal range and also pCO_2 was decreased as for coexisting respiratory alkalosis. Unfortunately, more detailed data are lacking, hence the existence of respiratory acid-base disorders in hemodialysis patients may be only conjectured. This notwithstanding, it should be acknowledged that Yamamoto *et al*[5] moved the spotlight away from serum HCO_3 concentration.

Finally, in a much smaller cohort of patients we have reported the prevalence of all kinds of simple and mixed acid-base disorders[6]. As expected, metabolic acidosis was the most common acid-base disorder. It was observed that metabolic acidosis as simple disorder was found in 38.7% of measurements and was coupled with respiratory acid-base disturbances in further 23.2%. The latter, as simple or complex disorders, were found in 41% of analyzed blood samples. This finding might be surprising, but the large and growing prevalence in hemodialysis patients of heart[7] and lung diseases[8] - known to be possibly associated with respiratory acidosis and alkalosis - accounts for such results. It should be needless to say that to characterize acid-base pattern of hemodialysis patients, as well of all other patients, pCO_2 should always be measured; however, here we want to emphasize that looking at HCO_3 concentration alone is not enough. This is not an academic issue, because a superimposing lung or heart disease can move up or down HCO_3 concentration toward the lower risk zone, but mortality risk of hemodialysis patient likely increases rather than decreases. Even though these results need further confirmation, the era of blood gas measurements in hemodialysis patients begins, and it perhaps occurs with some delay.

In conclusion, CO_2 as respiratory component of acid-base pattern is at least as important as the metabolic component in acid-base assessment also in hemodialysis patients.

Expected pCO_2 in metabolic acidosis

Metabolic acidosis is the commonest acid-base disorder occurring in hemodialysis patients[9-11]. Often it results in acidemia and consequently in increased ventilation to keep pH close to normal. As a result, pCO_2 decreases, and the magnitude of this reduction is closely dependent on how much serum HCO_3 concentration is decreased. Clearly, concurrent respiratory disorders may affect ventilatory compensation to metabolic acidosis, but this issue never received attention in this population. However mixed acid-base disorders - *i.e.,* respiratory acid-base disturbances superimposing to metabolic disorder - likely occur and, according to recent reports[6], they are not a rare occurrence. This finding is all but unexpected, as these patients carry a burden of heart and lung comorbid

conditions[2]. Accordingly, mixed disorders deserve full and prompt recognition, also in hemodialysis patients. To infer and diagnose mixed acid-base disorders clinicians must first evaluate the physiologic respiratory response to metabolic acidosis, namely they must estimate the value of partial pressure of pCO_2 complying with the reduced HCO_3 concentration. Ventilatory response to chronic metabolic acidosis is very predictable indeed; if the measured pCO_2 value is greater or lower than the computed "expected" one, then the presence of a mixed disorder can be inferred. Ventilatory response to chronic metabolic acidosis is independent of the disease causing acid-base derangement[12], hence rules for the general population and all other patient also apply to hemodialysis population. However, in textbooks[13-15] and in current literature[10,16] more than one formula and rule are available, but recommendations on what should be used are lacking. As formulas are different each other, results are often inconsistent; this notwithstanding, selecting the proper formula, *i.e.*, computing the proper value - is mandatory, to avoid wrong diagnosis and inappropriate treatment.

According to the long-lasting and widely used Winters' formula[17,18] pCO_2 can be predicted as serum $HCO_3 \times 1.5 + 8$. This formula was derived by Albert, Dell and Winters in the 60' in patients with severe acidosis and nowadays is still recommended, even though it lacks at all of any validation in patients with minor reductions of HCO_3 concentration. Intuitively, a slight reduction of HCO_3 is consistent with minor activation of the compensatory mechanisms whereas sizable decrease of serum HCO_3 elicits large increase of ventilation, hence a linear relationship - as Winters' formula is - might be not reliable throughout the acidosis spectrum.

Taking into account that serum HCO_3 in modern hemodialysis patients ranges around 20 mmol/L[2,3,6,11] which is exactly twice the mean value in Albert's population[17] - applying Winters' formula in this scenario is at least questionable. Even though it is recommended across-the-board to apply Winters' formula to hemodialysis population, that was associated with a larger error in prediction than other formulas.

A reliable alternative may be the common practical rule that reads "the reduction of pCO_2 with respect to its normal value equals 1.2 multiplied by the reduction of bicarbonate with respect to its normal value"[11,12,15,16]. This rule reliably predicts pCO_2 in mild-to-moderate acidosis; as a matter of fact, it has always been adopted in hemodialysis population[11,10]. If 40 mmHg and 24 mmol/L are the normal values of pCO_2 and of HCO_3, respectively, the rule can be read as $pCO_2 = 40 - (24\text{-}HCO_3) \times 1.2$ and equivalently rewritten as $pCO_2 = 1.2 \times HCO_3 + 11.2$. Besides, it requires quite a few computations - and therefore the label practical is not very fitting - also this rule is a linear relationship between pCO_2 and HCO_3, hence it cannot be conveniently applied to all degrees of severity of metabolic acidosis.

In this case the slope of linear equation is reduced to 1.2. The use of different multipliers for acidosis of different degree fulfills the concept that activation of compensatory mechanisms is gradual and progressive, hence non-linear. In other words ventilatory compensation to chronic metabolic acidosis varies with severity of acidosis and a quadratic or cubic equation, *i.e.*, a curve, better depicts the whole relationship between pCO_2 and HCO_3[12].

Unfortunately, this is an unfeasible option for physicians. However, as Bushinsky *et al*[12] highlighted, by restricting the analysis to HCO_3 values below 10 mmol/L ventilatory response can be predicted with good approximation by the linear equation with a slope equal to 1.5 - just the multiplier of Winters' formula - whereas if HCO_3 values range between 10.1 and 24 mmol/L the linear equation with a slope close to 1.2 - the multiplier of practical rule - allows to properly calculate the expected pCO_2 value. Accordingly, as we already suggested elsewhere[19,20], a reliable method to correctly predict pCO_2 may be the use of two different linear formulas depending on severity of metabolic acidosis (Figure 2).

Beyond the well-known and widely used above-mentioned formulas, several textbooks provide some tips to easy calculate the expected pCO_2. One of these rules - quite surprisingly - allows a very easy and valid prediction of pCO_2 value in hemodialysis population[19]. It simply suggests to add "15" to HCO_3 concentration to obtain the expected pCO_2 value, the so called "Bicarbonate plus 15" rule. With this very simple formula only 1 mmHg difference arises compared to practical rule when HCO_3 ranges between 14 and 24 mmol/L, as commonly occurs in almost all hemodialysis patients. In this population the very simple formula was associated with same (low) mean error exhibited by the practical rule (Table 1)[19] and therefore in this scenario it could be suggested as a valid and reliable alternative formula as it has the undeniable advantage of making CO_2 prediction easier and also attractive to physicians reluctant to approach the acid-base troubles.

CARBON DIOXIDE LOAD FROM DIALYSIS SOLUTION

Dialysis-related acidemia

The acid-base pattern of dialysate and of blood coming back from dialyzer to patient during bicarbonate hemodialysis has been recently recalled and has been labeled "dialysis-related acidemia"[21].

It has been above mentioned the compensatory response to metabolic acidosis that ultimately leads to hypocapnia - a common feature of hemodialysis patient - here we want to recall that pCO_2 in the final diluted dialysate is two-to-three folds the quantity found in the uremic blood entering the extracorporeal circuit. This large dialysate-blood difference accounts for very high CO_2 dialysance and in turn for the sizeable transfer of CO_2 from dialysate into the blood coming back to patient[22]. Even though high HCO_3 concentration, blood reaching patient's bloodstream is featured by low pH

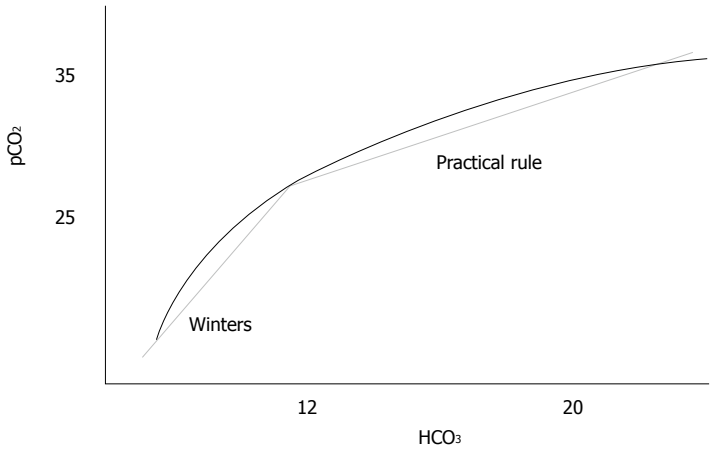

Figure 2 Relationship between pressure of carbon dioxide and bicarbonate concentration in chronic metabolic acidosis. The relationship between pCO_2 and HCO_3 during metabolic acidosis is graphically depicted as a curve. Two linear approximations (straight lines) equivalent to Winters' formula ($pCO_2 = 1.5 \times HCO_3 + 8$) and to the practical rule ($pCO_2 = 1.2 \times HCO_3 + 11.2$) are also shown. HCO_3: Bicarbonate concentration; pCO_2: Pressure of carbon dioxide.

Table 1 Errors in prediction of the expected pressure of carbon dioxide in hemodialysis patients

	Blood samples featuring $HCO_3 < 24$ mmol/L	Blood samples claimed for metabolic acidosis
"Winters' formula" $pCO_2 = 1.5\ HCO_3 + 8$	4.85	2.06
"Practical rule" $pCO_2 = 1.2\ HCO_3 + 11.2$	3.14	1.50
"Very simple formula" $pCO_2 = HCO_3 + 15$	3.09	1.56

Errors (root mean square errors in mmHg) in computing the expected pCO_2 in a dataset of blood gas measurements from chronic hemodialysis patients. Reproduced with permission from Ref. [19]. HCO_3: Bicarbonate concentration; pCO_2: Pressure of carbon dioxide.

due to very high pCO_2[22,23]. This pattern looks like respiratory acidosis but it has nothing to do with the lung. Moreover in hypercapnic acidosis partial pressure of oxygen (pO_2) is always decreased, whereas in dialysis-related acidemia does not, because a gain of oxygen across the filtering membrane also occurs. Dialysis-related acidemia vanishes as soon as CO_2 is breathed away by lung (hyper)ventilation, thus HCO_3 coming from dialyzer counteracts uremic acidosis. The source of CO_2 is dialysate itself, indeed mixing acid concentrate with HCO_3-containing solution the acid - commonly acetic acid - reacts with buffer leading to acetate anion and CO_2. The more the acid in acid concentrate, the more the CO_2 in the final diluted dialysate. As a typical example 3 mmol/L of acetic acid (or a mixture of citric and acetic acid) are in the concentrate and as a result 3 mmol/L of CO_2 are in dialysate. This leads to pCO_2 ranging between 80 and 100 mmHg and in turn to dialysate pH lower than 7.30. This allows calcium and magnesium bicarbonate salts to remain in their soluble form. The presence of CO_2 is actually mandatory and in the same way "an adequate ventilatory capacity is imperative to excrete the excess CO_2 generated during high efficiency bicarbonate hemodialysis"[23].

Acidosis by dialysate

If patients are unable to increase their ventilatory rate and in turn to breath away CO_2 overload from dialysate, then systemic pCO_2 increases leading to reduction of peripheral vascular resistance[24,25], harmful hypotension and severe dyspnea poorly relieved by oxygen administration for the time being. Dialysis treatment should be slowed down or even stopped to avoid more severe effects. As hypercapnia superimposes to metabolic acidosis, a mixed (metabolic plus respiratory) acidosis occurs with abrupt fall of blood pH. Hypoxia is only a later event. A few of such cases are reported in the literature[26-28], likely due to poor awareness of the syndrome, recently labeled "acidosis by dialysate"[21].

The burden of CO_2 in renal replacement therapies

The issue of CO_2 load during renal replacement therapy has been for long time neglected and has not in depth investigated. However theoretical considerations and some findings from literature allow to briefly comment on.

Acetate-free hemodiafiltration is an alternative dialysis technique claimed for allowing better hemodynamic stability and paucity of dialysis-related symptoms. It is featured by lack of any buffer in dialysate, indeed any acid is needed. Accordingly, the final diluted dialysate is "CO_2-free" other than "acetate-free" and this represents an important difference between acetate-free biofiltration and all other dialysis techniques. Even though some amount of CO_2 comes back to patients from sodium bicarbonate infusion, acetate-free biofiltration should be claimed for providing a lighter CO_2 load compared to conventional bicarbonate hemodialysis[29]. Outstandingly, pCO_2 in blood from dialyzer is very close to physiological amount, meaning that AFB might be suggested as the more advisable technique for patients unable to handle CO_2 overload as those with chronic obstructive lung disease, an increasingly prevalent comorbid condition[8].

On the other side online hemodiafiltration - regarded

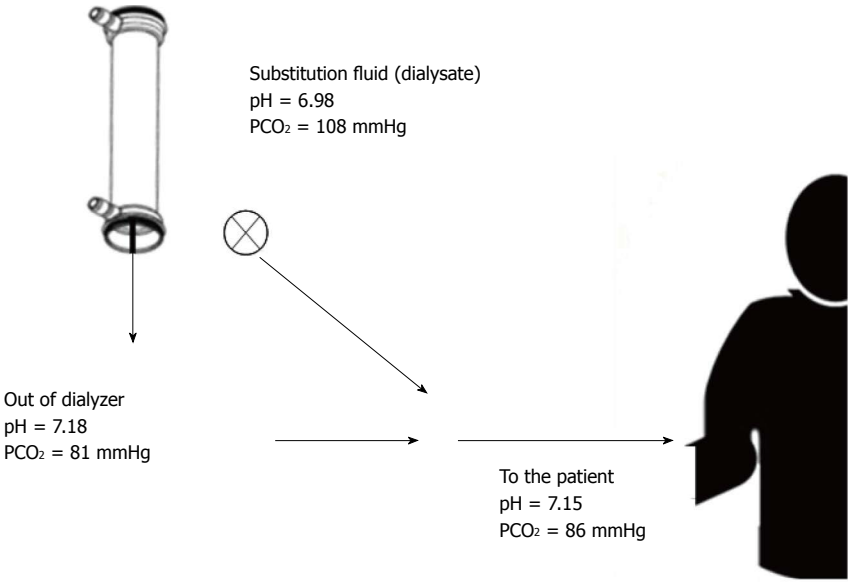

Figure 3 Example of gas analysis in on-line hemodiafiltration. Additional CO_2 load delivered *via* substitution fluid infusion during online hemodiafiltration. Reproduced with permission from Marano *et al*[30]. CO_2: Carbon dioxide.

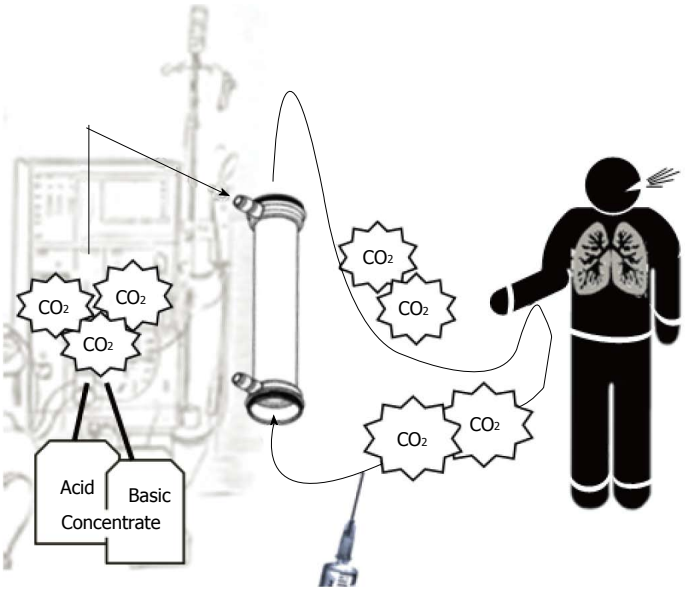

Figure 4 Course of carbon dioxide in presence of vascular access recirculation. pCO_2 from dialyzer re-entering the extracorporeal circuit reveals vascular access recirculation. pCO_2: Pressure of carbon dioxide.

as the new gold standard of renal replacement therapy - implies an heavier CO_2 load than bicarbonate hemodialysis does[30]. An additional CO_2 load is delivered by infusing dialysate, with its burden of CO_2, directly in patient's bloodstream (Figure 3). As the largest infusion volume possible has been recommended[31], the issue of CO_2 overload during online hemodiafiltration should be taken in account. Whether different CO_2 loads should be taken in account to withhold or in the opposite to recommend a certain replacement therapy to a certain hemodialysis patient is a question never asked.

RecirCO2lation test

If CO_2-enriched blood coming from the dialyzer reenters extracorporeal circuit, then vascular access recirculation may be detected by means of gas analysis of blood withdrawn from arterial line[32] (Figure 4). The typical acid-base picture of blood out the dialyzer - "dialysis-related acidemia" - is actually found in arterial line. As hypercapnic acidosis is coupled with normal or high pO_2, this acid-base pattern is unique and it is not suggestive of any human illness. Accordingly, vascular access recirculation may be easy and profitably discovered by means of easy blood sampling from arterial line of dialysis circuit. A pCO_2-increase > 4.5 mmHg (with respect to pre-dialysis value: "two samples technique") discovers vascular access recirculation with absolute specificity (100%) and high sensitivity (86.7%). A reliable alternative chance ("one sample technique") consists of a single blood sampling (5 min from dialysis start) to check whether pCO_2 is over or below a certain threshold. For both approaches, receiver operating characteristic analysis showed remarkable areas under curves (Figure 5). As a special feature of this novel test - labeled "RecirCO2lation test" - the use of CO_2 as indicator offers the undeniable chance of overcoming the issue of cardiopulmonary recirculation, because the excess of CO_2 coming from the dialyzer is time by time cleared away by

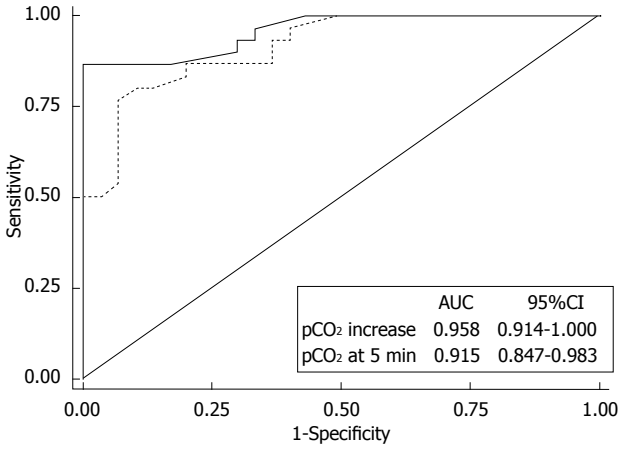

Figure 5 Performance of "RecirCO$_2$lation" test to detect vascular access recirculation. Receiver operating curves of pCO$_2$-increase (solid line) and pCO$_2$ at 5 min (dotted line) for diagnosis of vascular access recirculation using Glucose Infusion Test (> 0.3%) as reference. Reproduced with permission from Marano *et al*[32]. AUC: Area under curve; pCO$_2$: Pressure of carbon dioxide.

lungs and therefore if recirculation does not occur, it can never reaches arterial line.

CONCLUSION

CO$_2$ as respiratory component of acid-base pattern is at least as important as the metabolic component in acid-base assessment also in hemodialysis patients. To infer and diagnose mixed acid-base disorders, physiologic respiratory response to metabolic acidosis should be considered and the expected pCO$_2$ value should be computed. To do it, a very simple formula - "bicarbonate plus 15" - is a reliable alternative to the common practical rule, not so practical.

The acid-base pattern of blood coming back from dialyzer to patient during bicarbonate hemodialysis is featured by low pH due to very high pCO$_2$. Increasing ventilation rate is mandatory to excrete CO$_2$ overload, otherwise harmful "acidosis by dialysate" may occur. Among renal replacement therapies, acetate-free biofiltration is featured by a more physiological load of CO$_2$, whereas online hemodiafiltration implies an additional CO$_2$ load.

Finally, vascular access recirculation may be detected by means of gas analysis performed on blood withdrawn from arterial line of extracorporeal circuit. This novel method has been labeled "RecirCO$_2$lation test".

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ORIGINAL ARTICLE

Retrospective Cohort Study

Parathyroid ultrasonography and bone metabolic profile of patients on dialysis with hyperparathyroidism

Cláudia Ribeiro, Maria Goretti Moreira Guimarães Penido, Milena Maria Moreira Guimarães, Marcelo de Sousa Tavares, Bruno das Neves Souza, Anderson Ferreira Leite, Leonardo Martins Caldeira de Deus, Lucas José de Campos Machado

Cláudia Ribeiro, Center of Nephrology, Santa Casa de Belo Horizonte Hospital, Belo Horizonte CEP 30150320, Minas Gerais, Brazil

Maria Goretti Moreira Guimarães Penido, Marcelo de Sousa Tavares, Pediatric Nephrology Unit, School of Medicine, Federal University of Minas Gerais, Belo Horizonte CEP 30130100, Minas Gerais, Brazil

Maria Goretti Moreira Guimarães Penido, Marcelo de Sousa Tavares, Pediatric Nephrology Unit, Center of Nephrology, Santa Casa de Belo Horizonte Hospital, Belo Horizonte CEP 30150320, Minas Gerais, Brazil

Milena Maria Moreira Guimarães, Lucas José de Campos Machado, Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte CEP 30130100, Minas Gerais, Brazil

Bruno das Neves Souza, Leonardo Martins Caldeira de Deus, School of Medicine, Federal University of Minas Gerais, Belo Horizonte CEP 30130100, Minas Gerais, Brazil

Anderson Ferreira Leite, Department of Cardiology, Clinic Hospital, Federal University of Minas Gerais, Belo Horizonte CEP 30130100, Minas Gerais, Brazil

Author contributions: Ribeiro C, Penido MGMG, Guimarães MMM, Tavares MS and Machado LJC contributed equally to the conception and design of the study, the acquisition, analysis and interpretation of data, and the drafting and critical revision of the article; Souza BN, Leite AF and de Deus LMC performed the research and analyzed the data.

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Correspondence to: Maria Goretti Moreira Guimarães Penido, MD, PhD, Coordinator, Pediatric Nephrology Unit, Center of Nephrology, Santa Casa de Belo Horizonte Hospital, Rua Piaui 420, Belo Horizonte CEP 30150320, Minas Gerais, Brazil. mariagorettipenido@yahoo.com.br
Telephone: +55-31-99291595
Fax: +55-31-32414466

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Abstract

AIM

To evaluate the parathyroid ultrasonography and define parameters that can predict poor response to treatment in patients with secondary hyperparathyroidism due to renal failure.

METHODS

This cohort study evaluated 85 patients with chronic kidney disease stage V with parathyroid hormone levels above 800 pg/mL. All patients underwent ultrasonography of the parathyroids and the following parameters were analyzed: Demographic characteristics (etiology of chronic kidney disease, gender, age, dialysis vintage, vascular access, use of vitamin D), laboratory (calcium, phosphorus, parathyroid hormone, alkaline phosphatase, bone alkaline phosphatase), and the occurrence of bone changes, cardiovascular events and death. The χ^2 test were used to compare proportions or the Fisher exact test for small sample frequencies. Student *t*-test was used to detect differences between the two groups regarding continuous variables.

RESULTS

Fifty-three patients (66.4%) had parathyroid nodules with higher levels of parathyroid hormone, calcium and phosphorus. Sixteen patients underwent parathyroidectomy and had higher levels of phosphorus and calcium × phosphorus product (P = 0.03 and P = 0.006, respectively). They also had lower mortality (32% *vs* 68%, P = 0.01) and lower incidence of cardiovascular or cerebrovascular events (27% *vs* 73%, P = 0.02). Calcium × phosphorus product above 55 mg^2/dL^2 [RR 1.48 (1.06, 2.08), P = 0.03], presence of vascular calcification [1.33 (1.01, 1.76), P = 0.015] and previous occurrence of vascular events [RR 2.25 (1.27, 3.98), P < 0.001] were risk factors for mortality in this population. There was no association between the occurrence of nodules and mortality.

CONCLUSION

The identification of nodules at ultrasonography strengthens the indication for parathyroidectomy in patients with secondary hyperparathyroidism due to renal failure.

Key words: Secondary hyperparathyroidism; Parathyroid ultrasonography; Calcium; Phosphorus; Parathyroid hormone; Alkaline phosphatase; Chronic kidney disease; Bone alkaline phosphatase

Core tip: We aimed to evaluate the parathyroid ultrasonography and defined parameters to predict poor response to treatment in 85 patients with chronic kidney disease stage V and parathyroid hormone (PTH) levels > 800 pg/mL. Fifty-three patients had nodules, higher PTH, calcium and phosphorus. Sixteen underwent parathyroidectomy and had significant higher levels of phosphorus and calcium phosphorus product; lower mortality and lower incidence of cardiovascular or cerebrovascular events. Calcium phosphorus product above 55, vascular calcification and previous vascular events were risk factors for mortality. There was no association between nodules and mortality. We concluded that nodules at ultrasonography strengthens the indication for parathyroidectomy in those patients.

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INTRODUCTION

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a growing health care concern associated with secondary hyperparathyroidism (SHPT), mineral abnormalities and increased risk of cardiovascular disease[1]. Therefore, prevention and control of SHPT is one of the main objectives in the management of chronic kidney disease patients, particularly those on dialysis.

SHPT develops from the early stages of CKD due to disturbances in calcium (Ca), phosphorus (P) and vitamin D metabolism, characterized usually as high turnover bone disease[2]. Besides stimulating the synthesis and secretion of parathyroid hormone (PTH), hyperplasia of parathyroid cells, initially diffuse and then nodular[3], can also be observed. Patients with nodular parathyroid hyperplasia exhibit reduced number of Ca-sensing receptor (CaSR) and vitamin D receptor (VDR), and they are often resistant to vitamin D therapy[3,4]. In early stages of CKD, even without persistent hyperphosphatemia, elevated net body P load is associated with increased production of fibroblast-growth-factor 23 (FGF23) by bone osteoblasts and osteocytes. Serum FGF23 correlates to PTH in predialysis CKD patients and in patients with early CKD when levels of P and Ca are maintained within normal range[5-7]. However, in advanced stages of CKD, there is an inability of FGF23 to suppress PTH secretion.

A total transient elevation in P levels may be associated with reduction of serum Ca and thereby stimulate the secretion of PTH (tradeoff theory). Theories suggest that the hyperfiltration of the remaining nephrons could raise the P concentration in the proximal tubule cells, inhibiting the 1-alpha-hydroxylase. Experimental studies have shown a direct effect of P in increased PTH secretion[5] and have also demonstrated an independent association between phosphatemia and PTH in CKD patients[7,8].

Reduction of CaSR and VDR expression in parathyroid

cells occurs with the progression of CKD[9,10]. This process is more prominent in patients with nodular parathyroid hyperplasia than those with diffuse hyperplasia[9,11-13]. However, it is unclear why diffuse parathyroid hyperplasia develops to nodular hyperplasia. In this kind of hyperplasia (nodular hyperplasia), the parathyroid gland contains nodules of rapidly proliferating parathyroid cells. Each nodule, formed by monoclonal proliferation of parathyroid cells, produces excessive amounts of PTH. Because of the low density of CaSR and VDR, these cells are refractory to medical treatment (activated vitamin D, calcimimetics, control of hyperphosphatemia). During the progression of parathyroid hyperplasia, the ability of PTH synthesis increases progressively while a quantitative reduction of VDR and CaSR occurs, resulting in autonomous function of this gland, which characterizes the tertiary hyperparathyroidism[9,13,14].

The Kidney Disease Outcomes Quality Initiative proposes strict targets for the control of PTH, Ca and P in CKD patients[15] considering studies showing that patients with serum PTH levels above 800 pg/mL have severe and refractory hyperparathyroidism[14]. Considering that parathyroid hyperplasia is resistant to the action of vitamin D, it is important to identify among CKD patients those with high PTH levels and low response to this treatment, since they may be candidates for parathyroidectomy (PTX) in case of hypercalcemia (Ca > 10 mg/dL) and/or hyperphosphatemia (P > 5.5 mg/dL) in association with clinical symptoms, such as bone pain, fractures or spontan tendon rupture, untreatable chronic pruritus, erythropoiesis stimulating agents (ESA) resistant anemia without other causes and calciphylaxis[16,17].

Ultrasonography (US) is an economically feasible and noninvasive imaging method able to identify nodular hyperplasia[18,19] and serves as a marker of prognosis and response to treatment of SHPT with vitamin D analogues[20,21]. Increased parathyroids size, despite several aspects of the US and possible ectopic locations, are generally characterized by a distinct echogenic capsule, independent of the thyroid capsule, surrounding a hypoechoic area due to progressive hypercellularity and the disappearance of adipose tissue[22,23]. Gland weighing more than 0.5 g (equivalent to 0.5 cm^3) or larger than 1.0 cm or greater in diameter correspond to nodular hyperplasia in more than 90% of cases[24].

Severe SHPT is associated with higher mortality in patients with moderate and advanced CKD[25] as well as with disorders of bone metabolism (especially hyperphosphatemia and increased Ca × P product), and worse prognosis in CKD. Elevated PTH is responsible for serious long-term consequences, such as renal osteodystrophy, vascular and valvular calcification, changes in myocardial structure and function, immune dysfunction and anemia unresponsive to ESA[26,27], bone pain, abnormal bone histology and fractures among patients with SHPT[28,29].

The present study aimed to evaluate the usefulness of US of parathyroids in CKD patients on hemodialysis as a predictor of clinical outcome in severe hyperparathyroidism and correlate these sonographic findings and data related to CKD-MBD with clinical, epidemiological, laboratory and mortality data and response to treatment. As secondary endpoint, the study aimed to investigate the association between PTX and mortality of patients with CKD and BMD.

MATERIALS AND METHODS

Patients

It was a cohort study of patients on hemodialysis regularly followed at the Nephrology Center of Santa Casa de Belo Horizonte, Brazil, from January 2005 to January 2009. Inclusion criteria were patients with CKD on hemodialysis, age equal to or older than 18 years and at least one measuring serum intact PTH above 800 pg/mL. Patients with severe neuropsychiatric disorders, anatomical and/or functional changes that would interfere with the US examination and patients acutely unstable, such as with uncontrolled infection, hemodynamic and/or metabolic instability, as well as those who refused to participate in the study were excluded.

During the clinical follow-up, patients were submitted to monthly clinical and laboratory examinations according to Resolution - No. 154 of June 15, 2004, the Brazilian National Health Surveillance Agency (ANVISA).

This study was submitted to and approved by the Research Ethics Committee of the Graduation Center of Santa Casa de Belo Horizonte, Minas Gerais, Brazil (011/2005), and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CKD patients received calcitriol according to resolution SES # 267 of the State Health Authority of the State of Minas Gerais, Brazil (Table 1).

In case of no response (reduction of 30%-50% of the initial PTH), patients received vitamin D analogues according to Table 2.

Vitamin D administration was withdrawn under the following conditions: Hypercalcemia (plasma Ca above 10.0 mg/dL), hyperphosphatemia (plasma P above 5.5 mg/dL), Ca × P product above 55 mg/dL or PTH less than 200 pg/mL.

The parathyroid US was performed by an examiner and a medical resident in training with a Siemens Versa Plus Sleep Line and 7.5 Mhz transducer. Both unaware of any patient data. The gland volume was considered increased when presented a dimension of 1.0 cm in any axis, or a volume higher than 0.5 cm[3,20,24].

Laboratory data were collected at Time 1, from January 2005 to January 2006, and at Time 2, from October 2008 to January 2009. The following parameters were evaluated: Total Ca, P, alkaline phosphatase (AP), bone specific AP (BAP) at Time 1 only, PTH and Ca × P product. Blood samples were collected immediately before the first dialysis session of the week. PTH was measured by chemoluminescence and bone AP by the method of thermal inactivation.

The clinical and demographic data were: Age, sex,

Table 1 Initial dose of vitamin D analogue (calcitriol) to treat secondary hyperparathyroidism due to chronic kidney disease

PTH level	Vitamin D analogue dose (calcitriol)
PTH 200-499 pg/mL	0.25 mcg qd once a day
PTH 500-999 pg/mL	0.50 mcg qd or 1 mcg qd once a day/3 times a week
PTH > 1000 pg/mL	2.0 mcg qd once a day/3 times a week

Source: Minas Gerais Health Authority (Secretaria do Estado da Saúde de Minas Gerais). Available from: URL: http://www.saude.mg.gov.br/atos_normativos/resolucoes/2003. PTH: Parathyroid hormone.

Table 2 Mantainance dose of vitamin D analogue (calcitriol) to treat secondary hyperparathyroidism due to chronic kidney disease in initially unresponsive patients

PTH level	Vitamin D analogue dose (calcitriol)
PTH 200-299 pg/mL	0.25 mcg qd once a day
PTH 300-399 pg/mL	0.50 mcg qd or 0.75 mcg qd once a day, 3 times a week
PTH 400-999 pg/mL	1.0 mcg qd once a day 3 times a week
PTH > 1000 pg/mL	2.0 mcg oral or IV 3 times a week

Source: Minas Gerais Health Authority (Secretaria do Estado da Saúde de Minas Gerais). Available from: URL: http://www.saude.mg.gov.br/atos_normativos/resolucoes/2003. PTH: Parathyroid hormone; IV: Intravenous.

time on hemodialysis, CKD etiology, type of access for hemodialysis and the presence of bone and/or vascular changes detected by imaging method. Figure 1 summarizes the patients selection and follow-up.

Statistical analysis

The two groups (with and without nodules) were subjected to a descriptive analysis, and nominal/categorical variables as well as frequency distribution were represented in tables. Continuous variables and measures of central tendency and variability were used.

Univariate analysis of categorical variables were performed with the χ^2 test to compare proportions or the Fisher exact test when small sample frequencies were used. Student *t*-test was used to detect differences between the two groups regarding continuous variables. In all analyzes a significance level of 5% level was considered and SPSS v. 12.0 software was used for the analysis.

RESULTS

Patient characteristics

From January 2005 to January 2006 (Time 1), the 85 patients with PTH levels above 800 pg/mL underwent US parathyroid glands, 53 (62.4%) had at least one nodule and 32 (37.6%) showed no abnormality.

Mean age was 44.5 ± 13 years (range 18-76 years), 52% were female. Mean time on hemodialysis in patients without nodules (104.4 ± 54 mo) was similar to the

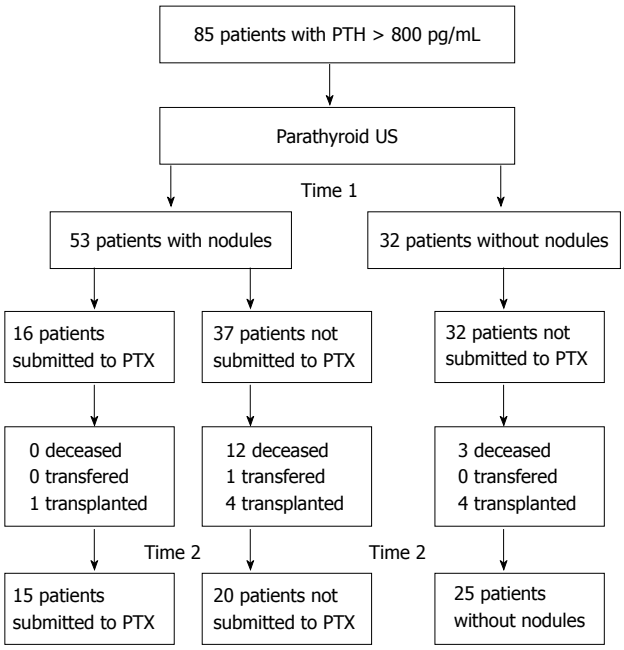

Figure 1 Fluxogram of follow-up of 85 pacientes with chronic kidney disease stage V submitted to ultrasonography of parathyroids between January 2005 to January 2006 (Time 1) and then until January 2009 (Time 2). PTH: Parathyroid hormone; PTX: Parathyroidectomy; US: Ultrasonography.

group with parathyroid nodules (106 ± 65 mo; $P = 0.91$). There was no difference between groups regarding the type and dialysis access. Most patients were on hemodialysis with arteriovenous fistula as vascular access (89.4%, $P = 0.47$) (Table 3). Glomerular and vascular diseases were the most common causes of CKD in both groups.

Regarding the use of vitamin D-analogues, 6 patients (7%) did not follow the treatment, and four failed to achieve adequate levels of P and two did not achieve adequate levels of both Ca and P, which did not allow the treatment of SHPT. There was no difference between the groups regarding the use of calcitriol ($P = 0.402$). Patients with parathyroid nodules had higher PTH, Ca, P and Ca × P product. There was no significant difference between the levels of AP and BAP (Table 4).

There was no difference between groups when outcomes were analyzed, considering the 16 patients who underwent PTX in this period (Table 5).

At Time 2 (October 2008 to January 2009), 60 patients remained in the study; 9 were transplanted, 15 deceased and one was transferred to another facility. In this period there was no change regarding PTH, Ca, P, Ca × P and AP between the groups with and without nodules (Table 6).

In the comparison between Times 1 and 2, there was no difference in any variable in the group without nodule. In the group with nodules, there was a reduction in the levels of P (5.8 *vs* 5.1, $P = 0.008$ by paired *t* test) and Ca × P product (51.6 *vs* 44.6, $P = 0.007$ by paired *t* test).

At Time 1, there was a significant difference in AP and BAP variables, with higher values in the group

Table 3 Clinical and demographical characteristics of the studied population

	Without nodule (n = 32)	With nodule (n = 53)	Total	P	RR (95%CI)
Sex					
Male	16	25	41	0.80[1]	
Female	16	28	44		1.12 (0.46-2.69)
Total	32	53	85		
Age	43 ± 14	46 ± 13		0.18[2]	1.02 (0.99-1.06)
Time on dialysis	104.4 ± 53.6	106.0 ± 64.7		0.91[2]	1.00 (0.99-1.01)
Access for RRT					
Others	2	7	9	0.47[1]	2.3 (0.4-11.7)
AVF	30	46	76		1.00

[1]Pearson χ^2 test; [2]t-Student test. AVF: Arteriovenous fistula; RRT: Renal replacement therapy.

Table 4 Comparison of laboratory results of patients without and with nodule

Surgery	PTH	Ca	P	Ca × P	AP	BAP
No nodule (n = 32)	796.2 ± 229.7	8.6 ± 0.7	4.8 ± 1.2	41.5 ± 12.4	246.7 ± 128.7	100.8 ± 69.6
With nodule (n = 53)	1106.4 ± 462.5	9.0 ± 0.6	5.9 ± 1.0	53.1 ± 10.3	283 ± 254.4	106.4 ± 91.9
P	< 0.001	0.006	< 0.001	< 0.001	0.387	0.783

Student's t-test. PTH: Parathyroid hormone; BAP: Bone specific alkaline phosphatase.

Table 5 Observed outcomes until end of follow-up in January 2009 (n = 85)

	Nodule in US		P	RR (95%CI)
	No (n = 32)	Yes (n = 53)		
Deceased				
No	29 (90.6%)	41 (77.4%)	0.120[2]	1.00
Yes	3 (9.4%)	12 (22.6%)		2.83 (0.73; 10.93)
Transplant				
No	28 (87.5%)	48 (90.6%)	0.723[2]	1.37 (0.54; 5.53)
Yes	4 (12.5%)	5 (9.4%)		1.00
Cardiovascular and/or cerebrovascular event[4]				
No	25 (78.1%)	43 (81.1%)	0.737[1]	1.20 (0.41; 3.56)
Yes	7 (21.9%)	10 (18.9%)		1.00
Vascular calcification[5]				
No	23 (71.9%)	33 (62.3%)	0.633[1]	1.00
Yes	7 (21.9%)	13 (24.5%)		1.29 (0.40; 4.28)
No register	2 (6.3%)	7 (13.2%)		
Bone disease[6]				
No	18 (56.3%)	34 (64.2%)	0.202[1]	1.89 (0.63; 5.56)
Yes	12 (37.5%)	12 (22.6%)		1.00
No register	2 (6.3%)	7 (13.2%)		
Surgery				
No	32 (100%)	37 (69.8%)	< 0.001	[3]
Yes	0 (0%)	16 (30.2%)		

[1]Pearson's χ^2; [2]Fisher's exact test; [3]It was not possible to calculate OR due to null cases; [4]Patients' records: Acute myocardial infarct, acute coronary syndrome, unstable angina, acute arterial occlusion, congestive heart failure, abnormal findings in heart catheterism, coronary angioplasty and myocardial and coronary angioplasty and myocardial revascularization; [5]Vascular calcification in imaging (ultrasonography, computed tomography or plain radigraphies); [6]Imaging or notes on them, with evidence of bone disease attributed to SHPT, and bone deformities or fractures attributed to the disease. US: Ultrasonography; SPTH: Secondary hyperparathyroidism.

submitted to PTX (Table 7). At Time 2, they got higher and lower values of P and Ca × P product, as well of AP, compared to non-operated group (Table 8).

Comparing Time 1 with Time 2, in patients with nodular and non-operated, there were elevated levels of Ca (8.7 *vs* 8.9, P = 0.033 by Mann-Whitney test). Those submitted to PTX, a reduction of Ca, P and Ca × P product was observed (Table 9).

In relation to outcomes, a higher incidence of death and vascular events in patients with nodules not submitted to PTX was observed. Patients who died by the end of the study had higher mean values of Ca, P

Table 6 Comparison of parathyroid hormone, calcium, phosphorus, calcium × phosphorus, alkaline phosphatase between October 2008 and January 2009 for each group (n = 60)

Nodules		PTH	Ca	P	Ca × P	AP
Without nodules (n = 25)	Mean	833.2	8.70	4.80	41.4	249.6
	SD	602.2	0.7	1.40	12.50	255.6
With nodules (n = 35)	Mean	1031.2	8.70	5.10	44.60	396.2
	SD	775.1	0.9	1.50	13.60	535.8
	P^1	0.271	0.974	0.341	0.350	0.210

[1]Student's t-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase.

Table 7 Comparison between patients submitted or not to parathyroidectomy

Surgery	PTH	Ca	P	Ca × P	AP	BAP
No (n = 37)	1030.8 ± 391.8	9.0 ± 0.6	6.0 ± 1.0	53.7 ± 10.9	212.6 ± 121.5	82.7 ± 55.3
Yes (n = 16)	1281.1 ± 571.4	9.1 ± 0.6	5.7 ± 1.0	51.6 ± 9.0	445.9 ± 385.1	160.5 ± 132
P^1	0. 125	0. 605	0. 354	0. 497	0. 030	0. 050

[1]Student's t-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase; BAP: Bone specific alkaline phosphatase.

Table 8 Comparison of results of parathyroid hormone, calcium, phosphorus, calcium × phosphorus product, alkaline phosphatase levels from October 2008 to January 2009, patients with nodules, with and without parathyroidectomy (n = 35)

Surgery		PTH	Ca	P	Ca × P	AP
No (n = 20)	Mean	992.4	8.90	5.60	49.90	214.9
	SD	639.5	0.80	1.20	11.70	251.7
Yes (n = 15)	Mean	1082.9	8.40	4.50	37.60	638
	SD	948.2	1.10	1.60	13.10	708.3
	P^1	0.752	0.126	0.030	0.006	0.041

[1]Student's t-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase.

Table 9 Comparison of laboratory results of patients with nodules submitted to parathyroidectomy (n = 15) in 2 different periods: 2005/2006 and 2008/2009

	2005/2006			2008/2009			P
	Mean	Median	SD	Mean	Median	SD	
PTH	1281.1	1155.7	571.4	1082.9	811.0	948.2	0.173[1]
Ca	9.1	9.1	0.7	8.4	8.7	1.1	0.017[1]
P	5.7	5.8	1.0	4.5	4.3	1.6	0.009[1]
Ca × P	51.8	53.0	9.3	37.6	37.6	13.1	0.004[1]

[1]Mann-Whtiney's test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus.

and Ca × P product at Time 1. Analysis of categorical variables, Ca × P product above 55 mg^2/dL^2 was associated with a 48% higher risk of death. Patients who experienced cardiovascular events and vascular calcification in imaging also had a higher risk of death. Those patients who died and had visible nodules in US, had higher mean values of Ca and Ca × P product at Time 1, but no demographic differences was observed in relation to those who did not die. In this group, patients who experienced cardiovascular and/or cerebrovascular events had almost 200% higher chance of death.

There was no difference in demographic and clinical characteristics between patients with and without nodule on US. At Time 1, patients with nodules had higher levels of Ca, P, Ca × P product and PTH. There was no difference in the occurrence of death, vascular events or bone disease. Patients with nodules submitted to surgery had longer time on dialysis. They also had significantly reduced levels of Ca, P and Ca × P product, without variation of PTH levels. Among patients with nodule, mortality and the occurrence of cardiovascular events was lower in those who underwent surgery. Those who died had higher levels of Ca, P and Ca × P product and highest occurrence of events and vascular

calcification.

DISCUSSION

The present study shows that the identification of parathyroid nodules on US may be an indication criteria for PTX in patients with severe hyperparathyroidism associated with CKD, because this surgery is associated with lower morbidity and mortality in these patients. This finding is highly relevant in view of the simplicity and low cost of US and the great difficulty the treatment of SHPT in CKD. Despite the extensive knowledge about its pathophysiology, treatment of SHPT is complex, involving many factors to achieve therapeutic success. In this sense, achieving optimal levels of Ca, PTH and targets have been a challenge for everyone. The Dialysis Outcomes and Practice Patterns Study, which involves European countries and the United States, shows a large percentage of patients with controlled SHPT[16].

The presence of parathyroid nodules is associated with poorer bone metabolic profile in patients with severe hyperparathyroidism. In fact, corroborating the observation of other researchers[30-34], in the first moment of our study (Time 1) the levels of PTH, Ca, P and Ca × P product were higher in patients with parathyroid nodules. Furthermore, we did not find significant differences related to sex, age, time on renal replacement therapy (RRT), underlying disease or vascular access between groups with and without nodules. This in part could be attributed to the high cut-off level of PTH (> 800 pg/mL) in our study. These data would predict an increased difficulty in achieving the therapeutic goal in patients with severe hyperparathyroidism, despite the use of vitamin D-analogues. In fact, experiments have shown that the increase in parathyroid predict correlates with resistance to therapy with vitamin D-analogues (p.o. or parenteral)[3,20,35].

However, the analysis of bone metabolic profile at Time 2 showed that our patients without nodules also demonstrated that resistance and did not show improvements in mean values of Ca, P and PTH. Non-pharmacological factors may have interfered as poor adherence, supply failures and errors in prescriptions and their interpretation, as well as the initial severity of bone metabolic disorder itself, suggested by the high cut-off level used as inclusion criteria. In fact, despite a better bone metabolic profile at Time 1, this initial severity is suggested by the same proportion of outcomes observed in patients with and without parathyroid nodules.

Nevertheless, the group with nodules showed a reduction of P and Ca × P product probably due to PTX. Patients undergoing PTX had a reduction of Ca, P and Ca × P product. Patients with nodules and non-submitted to surgery had increased Ca levels, while other parameters remanied stable. In any group a decrease of PTH was observed.

This observed differences concerning the osteo-metabolic profiles between the groups with and without parathyroid nodules cannot be attributed to a more severe osteometabolic disease observed in patients who died, as there was no difference in mortality between these groups. The declining profile among patients with nodules who were not operated, not observed among those without nodules, also not operated, suggests that this beneficial effect of PTX on bone metabolic profile in patients with severe hyperparathyroidism is more prominent and perhaps unique to patients with SHPT with nodules. In this sense, we must consider that PTX is the last therapeutical approach to severe SHPT, meaning there was clinical treatment failure. Patients undergoing PTX have high rates of early mortality, despite lower rates on long-term[36,37].

The PTX allowed an improvement of bone metabolic profile in our patients. It was not our objective to evaluate success of PTX rates, the surgical techniques used or pathology results. In general, studies show that different surgical techniques have different recurrence rates, persistence and varied complications. However, one study showed similar changes in postoperative patients undergoing total PTX with or without autograft, except for a greater need for Ca replacement[38]. Comparing patients with nodules, submitted or not to PTX, we observed that only dialysis vintage was different, higher in those submmited to PTX, with no difference concerning age, sex and type of vascular access. In other studies, different factors were associated to PTX: Younger age, female gender, non-diabetic etiology of CKD, longer dialysis vintage, parenteral use of vitamin D and peritoneal dialysis. Remarkable are younger age and the dialysis vintage[27,36,37,39]. In our study no predominance of a determined cause of CKD was observed, nor differences regarding the use of vitamin D analogues.

Our data showed that the group of patients with nodules was not associated with an increased risk of unfavourable outcome - death, or vascular events and bone changes or differences concerning the chance of transplantation. On the other hand, in the group with nodules not submitted to PTX, a higher mortality and further vascular events was observed. This could be attributed to a lower chance of surgery in patients with a critical state, who eventually died. However, patients with nodules and underwent surgery at Time 1 had poorer bone metabolic profile (higher levels of AP and BAP). The better metabolic control after surgery may have contributed to better survival and lower morbidity. In addition, other studies have shown that, in relation to clinical treatment, PTX is associated with higher early mortality (up to 6 mo), probably to a higher postoperative risk, but lower in the long-term, which could be explained by an improvement in the metabolic and cardiovascular profile with reduced vascular calcification in imaging methods[40,41]. There was also improvement in symptoms related to bone metabolic metabolism, among those submitted to surgery[42,43].

Patients who died in Time 1 had higher levels of Ca, P and Ca × P product. They did not differ, however, from those who survived regarding gender, age, dialysis

vintage, type of vascular access, treatment with vitamin D-analogues, presence of parathyroid nodules and PTH levels. Within the group of patients with nodules, those who died presented at Time 1 higher values of Ca × P product, but did not differ from those who survived concerning the other parameters. A higher risk of death was observed in patients with cardiovascular events or vascular calcifications. In the population with nodules, the occurrence of death was higher among those with vascular events during the study. There was also a higher incidence of death and vascular events in patients with nodules and not submitted to PTX. These data suggest that bone metabolic profile can be a predictor of death in patients with severe HPT.

We cannot affirm that the presence of parathyroid nodule can also be a predictor of death in patients with severe hyperparathyroidism. However, as the operated group, with worse metabolic profile had improved this profile and had better outcomes, there is the possibility that the presence of parathyroid nodules can also be predictor of death.

In fact, studies have shown increased risk of death with P levels above 5 and 6.5 mg/dL, Ca × P product > 72 mg^2/dL^2 and PTH > 600 pg/mL[44,45]. The most common cause of death among patients on RRT is cardiovascular, with cardiac calcification in 60% of them. Joins P mortality, cardiovascular morbidity and mortality and progression of renal disease with or without CKD[46,47]. Elevated phosphate induces calcification of smooth muscle cells (SMC) *in vitro*. Pit-1, a sodium-dependent phosphate cotransporter is essential for SMC calcification and phenotypic modulation in response to elevated phosphate. P induces the expression of bone markers and extracelular matrix mineralization[46]. Calciphylaxis is the worst expression of the phenotypic modulation.

There is a strong association between high levels of P, Ca × P product and PTH and cardiovascular morbidity and mortality. The main mechanisms involved in this process are accelerated calcification and atherosclerotic instability, Ca accumulation in the tunica media, hypertension, left ventricular (LV) hypertrophy by direct trophic effects and secondary hypertension and coronary heart disease, anemia, and macro/microangiopatias[48,49]. PTH has been implicated in abnormalities in CKD-MBD: Immune dysfunction, refractory anemia, lower secretion of insulin[27]. Even in patients without CKD and SHPT, PTH has been associated with higher mortality[50,51] and P linked to higher mortality as well, and LV hypertrophy, cardiovascular events and coronary calcification[52,53].

Although it seems obvious the association of high levels of Ca, P and PTH in several possible combinations and morbidity and mortality, mainly related to cardiovascular events, it is unclear how the PTX can reduce these rates. In our study patients submitted to surgery and with a 49 mo follow-up had lower mortality rates and reduced levels of Ca and P. However, the effect on PTH was not significative and there was no significant correlation between PTH and mortality. It is plausible to question the correspondance between the success of a PTX as measured by PTH fall, and, therefore, the clinical improvement of a population in which the surgery was not effective. Other unevaluated factors had probably played a role.

The hormone fibroblast growth factor (FGF23) was first described in 2000 and it is mainly produced by osteocytes as a protein that reduces serum P through downregulation of the Na-P-cotransporter type 2 and leads to inhibition of the synthesis of 1-alpha-hydroxylase, with consequent reduction of calcitriol and increased serum PTH levels. The increase of FGF23 occurs in early stages of CKD, even before changes in serum levels of P and PTH and has been an independent factor of mortality in this population. In patients on hemodialysis, FGF23 levels can predict refractory SHPT[54-56]. Although not evaluated in our study, FGF23 could have explained some issues raised by the results.

Our study has limitations. It was retrospective and based on not standardized data and medical records which may be critical to the results. Moreover, another criticism is due to the fact that none of the patients have used other vitamin D analogs, such as paricalcitol and calcimimetics, whose results have demonstrated better clinical response than with traditional analogs of vitamin D. Two large studies have shown good responses to reduce PTH levels but failed to demonstrate reduction of morbidity and cardiovascular mortality: The EVOLVE and the PRIMO[54-56]. The first was done with cinacalcet and the second with paricalcitol. Although calcimimetics have reduced the need for surgical PTX, a Cochrane recent review showed no reduction in mortality in the population with CKD stage V with the use of cinacalcet[57].

In conclusion, patients with PTH > 800 pg/mL and parathyroid nodule presented worse bone metabolic profile than those without nodules. Hypercalcemia, hyperphosphatemia and elevated Ca × P product were associated with higher mortality in this population and PTX was associated with improvements in the control of Ca, P levels and Ca × P product, a lower occurrence of vascular events and longer survival in patients with severe SHPT.

Thus, the presence of nodules on US could be used as a criterion for PTX indication in this group of patients. Furthermore, it is possible that the presence of parathyroid nodule at US may be useful for the prediction of mortality or vascular events in patients with PTH levels higher than 800 pg/mL, although our study did not show this association.

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COMMENTS

Background

Secondary hyperparathyroidism has a high prevalence in the chronic renal dialysis population as cause of cardiovascular morbidity and mortality. The presence of nodular hyperplasia in parathyroids in ultrasound could predict response to drug treatment and guides the surgical treatment.

Research frontiers

To identify prognostic factors of response to pharmacological treatment with non invasive tests, in order to propose new therapies or indicate surgical treatment.

Innovations and breakthroughs

Ultrasonography could be an adjuvant exam in the follow-up of patients with severe hyperparathyroidism, in order to plan the treatment.

Applications

This study confirms the role of secondary hyperparathyroidism in cardiovascular mortality in chronic kidney disease patients in dialysis, associated with bone mineral disorders.

Terminology

Secondary hyperparathyroidism is a hormonal disorder triggered by many metabolic abnormalities that are related to renal function impairment.

Peer-review

The authors support that "The identification of nodules at ultrasonography strengthens the indication for parathyroidectomy in patients with secondary hyperparathyroidism due to renal failure".

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ORIGINAL ARTICLE

Observational Study

Is overhydration in peritoneal dialysis patients associated with cardiac mortality that might be reversible?

Elizabeth Oei, Klara Paudel, Annemarie Visser, Hazel Finney, Stanley L Fan

Elizabeth Oei, Klara Paudel, Stanley L Fan, Department of Renal Medicine and Transplantation, Barts Health NHS Trust, London E1 1BB, United Kingdom

Annemarie Visser, Department of Nutrition and Dietetics, Barts Health NHS Trust, London E1 1BB, United Kingdom

Hazel Finney, Department of Clinical Biochemistry, Barts Health NHS Trust, London E1 1 BB, United Kingdom

Author contributions: Oei E and Fan SL designed the study, generated the tables and co-wrote the manuscript; Paudel K, Visser A and Finney H contributed to data collection and writing of the manuscript.

Institutional review board statement: The study (Is overhydration in peritoneal dialysis patients associated with cardiac mortality that might be reversible?) was conducted in accordance with the principles set out by the local ethical committee according to United Kingdom National Health Service audit and clinical service development.

Informed consent statement: The study (is overhydration in peritoneal dialysis patients associated with cardiac mortality that might be reversible?) was conducted in accordance with the principles set out by the local ethical committee according to United Kingdom National Health Service audit and clinical service development. Patient consent is not required for data to be included in an audit and clinical service development (Quality Assurance) assessment.

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Correspondence to: Dr. Stanley L Fan, Department of Renal Medicine and Transplantation, Barts Health NHS Trust, Whitechapel Road, Whitechapel, London E1 1BB, United Kingdom. s.fan@qmul.ac.uk
Telephone: +44-20-35942674
Fax: +44-20-35942691

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Abstract

AIM

To study the relationship between overhydration (OH) in peritoneal dialysis (PD) patients and cardiac mortality.

METHODS

OH, as measured by body composition monitor (BCM), is associated with increased mortality in dialysis patients. BCM has been used to guide treatment on the assumption that correcting OH will improve cardiac morbidity and mortality although data demonstrating causality that is reversible is limited. We wished to determine if OH in PD patients predicted cardiac mortality, and if there was a correlation between OH and cardiac troponin-T (cTnT) levels. Finally, we wished to determine if improving OH values would lead to a decrement in cTnT. All prevalent PD patients over the study period of 57 mo who had contemporaneous BCM and cTnT measurements were followed irrespective of transplantation or PD technique failure. We also studied a cohort of patients with who had severe OH (> +2L).

The Fresenius Body Composition Monitor was used to obtain hydration parameters. cTnT levels were done as part of routine clinical care. Data was analysed using SPSS version 20.0.

RESULTS

There were 48 deaths in the 336 patients. The patients that died from cardiac or non-cardiac causes were similar with respect to their age, incidence of diabetes mellitus, gender, ethnicity and cause of renal failure. However, the patients with cardiac causes of death had significantly shorter dialysis vintage (10.3 mo *vs* 37.0 mo, $P < 0.0001$) and were significantly more overhydrated by BCM measurement (2.95 L *vs* 1.35 L, $P < 0.05$). The mean (standard error of the means) hydration status of the 336 patients was +1.15 (0.12) L and the median [interquartile range (IQR)] cTnT level was 43.5 (20-90) ng/L. The cTnT results were not normally distributed and were therefore transformed logarithmically. There was a statistically significant correlation between Log (cTnT) with the OH value (Spearman *r* value 0.425, $P < 0.0001$). We identified a sub-group of patients that were severely overhydrated; median (IQR) hydration at baseline was +2.7 (2.3 to 3.7) L. They were followed up for a minimum of 6 mo. Reduction in OH values in these patients over 6 mo correlated with lowering of cTnT levels (Spearman *r* value 0.29, $P < 0.02$).

CONCLUSION

Patients that were overhydrated had higher cTnT, and had deaths that were more likely to be cardiac related. Reduction in OH correlated with lowering of cTnT.

Key words: Bioimpedance; Fluid status; Peritoneal dialysis; Mortality; Overhydration; Cardiac troponin

Core tip: Overhydration measured by bioimpedance spectroscopy is an independent predictor of death in peritoneal dialysis patients. Most studies on this topic provide only a single baseline bioimpedance assessment. We present longitudinal data showing increased cardiac mortality in overhydrated patients, and significant correlation of overhydration with cardiac troponin-T (cTnT) levels. Over 6 mo, these patients had a mean of 7.4 body composition monitor readings and 3.4 cTnT assessments. Patients whose hydration status improved showed a corresponding improvement in cTnT. While observational studies cannot define causality, our results show overhydration is associated with cardiac mortality, and suggest overhydration may be a reversible risk factor.

Oei E, Paudel K, Visser A, Finney H, Fan SL. Is overhydration in peritoneal dialysis patients associated with cardiac mortality that might be reversible? *World J Nephrol* 2016; 5(5): 448-454 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i5/448.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i5.448

INTRODUCTION

Fluid overload measured by bioimpedance spectroscopy (BIS) is an independent predictor of death in peritoneal dialysis (PD) patients[1] and is highly prevalent[2]. This was also shown in haemodialysis (HD)[3]; patients with severe overhydration (OH) had a hazard ratio for all-cause mortality of 2.1, second to only diabetes. However, causality cannot be determined from these retrospective observational studies. Nevertheless, there is circumstantial evidence to support the belief that correcting OH will improve patient outcomes; correcting OH was shown to be associated with improvements in blood pressure, arterial stiffness and left ventricular mass index[4,5]. But very strict attention to fluid restriction was also shown to increase loss of residual renal function[6] and there is an association between low hydration status and intra-dialytic hypotension[7].

While it is often assumed that the observed relationship between OH and mortality is related to cardiovascular damage, an important caveat is that the ratio of extracellular fluid to total body water is also increased in the setting of muscle wasting. Thus, a negative correlation between BIS-OH parameters and malnutrition have been found in several studies[8,9] and it is possible that the increased mortality associated with OH relate to its association with protein energy wasting (PEW)/malnutrition inflammation atherosclerosis (MIA) Syndrome. Thus, in a study of 72 stable HD patients, although N-Terminal pro-Brain Naturetic Peptide (NTpro-BNP) correlated with BIS OH, the authors concluded that NTproBNP was also elevated in malnourished patients[10]. Others have also expressed reservation about the proposed causal relationship between overhydration measure by BIS and mortality[11].

We wished to explore this subject further by determining if PD patients who died from cardiac causes were more severely OH compared to patients that died from other causes. We also wished to determine if there is a relationship between OH and cardiac troponin-T (cTnT) which remains a highly sensitive biomarker for cardiac injury in dialysis patients[12,13]. Thus, we studied patients over a 6-mo period to determine if severe OH improved, did it lead to a corresponding decrements of cTnT. We also examined the changes in cTnT over 6 mo against time-average values of biochemical nutrition parameters (serum albumin and haemoglobin) as well the inflammatory marker of C-reactive protein (CRP).

MATERIALS AND METHODS

The study was conducted in accordance with the principles set out by the local ethical committee according to United Kingdom National Health Service audit and clinical service development. We studied a cohort of patients from a single PD unit, consisting of all continuous ambulatory PD (CAPD) and automated PD (APD) patients between 1 January

2008 and 30 March 2012 who had contemporaneous baseline BIS/cTnT readings. All patients with amputations, cardiac pacemakers or defibrillators were excluded as we were unable to perform BIS measurements. Patients were followed up until 15th September 2012 or death. Patients who were transplanted or switched to HD were still followed up. Only patients that recovered renal function or who were transferred to another dialysis unit for geographic relocation reasons were censored at that time point, as their survival follow-up could not be accurately determined. In all cases, baseline characteristics were collated through review of case notes and included primary cause of renal failure, dialysis vintage and presence or absence of diabetes mellitus. To comply with the mandatory United Kingdom Renal Registry submissions, we held weekly multidisciplinary meetings to review and assign causes of death for dialysis patients.

In a subgroup analysis, we identified patients that were severely overhydrated (OH > 2 L). Using the time when their OH was first found to be > 2 L as baseline, we prospectively collected data on their hydration status and their cTnT readings over the subsequent 6 mo.

Bioimpedance analysis

The Fresenius Body Composition Monitor (BCM - Fresenius Medical Care, Bad Homberg, Germany) was used to obtain hydration parameters such as OH and nutritional indices, namely Fat and Lean Tissue Mass (FTM and LTM respectively). This BIS device employed 50 frequencies between 5 and 1000 kHz, and measurements were performed by placing electrodes on one hand and one foot, with PD dialysate *in situ*.

Cardiac troponint assay

Over the duration of the study, cTnT was measured every 3-4 mo as part of routine clinical care. During this study, the cTnT assay (Roche Diagnostics GmbH, Mannheim, Germany) was changed to the high sensitivity assay, though the upper limit of normal was the same (< 14 ng/L, upper 99th percentile). In patients with normal renal function, values > 3 ng/L were suggestive of myocardial injury and values > 14 ng/L were considered diagnostic for myocardial infarction if there were consistent clinical features. Precision of the assays across the range were: 0.5 ng/L (1.9%); 0.0399 (1.6%); 0.133 (1.7%).

Statistical analysis

Categorical variables are expressed as a number and a percentage. Continuous variables are expressed as means and standard error of the means (SEM) or median with quartile ranges depending on whether the results of the parameters were normally distributed (determined by the D'Agostino and Pearson omnibus normality test). If not normally distributed, these parameters were analyzed on a logarithmic scale. Correlation coefficients and multivariate logistical regression analyses were undertaken with SPSS software for Windows version 20.0 (SPSS Inc., Chicago, IL, United States). The regression model was created based on those clinical variables known to effect survival on PD.

RESULTS

Demographics

There were 336 APD and CAPD patients who had at least 1 contemporaneous BCM/cTnT assessment during the study period. The median age of patients was 57.9 [interquartile range (IQR): 47.9-69.0] years with a median dialysis vintage of 7.6 (IQR: 0.9-31.4) mo (Table 1). 62% were male, and 37% had diabetes mellitus.

There were 74 patients who had an OH reading > 2 L and a subsequent BCM/cTnT assessment between 6-9 mo later. We excluded 8 patients that had documented acute cardiac events (acute rise in cTnT associated with cardiac pain, pulmonary oedema or haemodynamic instability). For the remaining 66 patients, the median (IQR) "baseline" OH value was 2.7 (2.3-3.7) L. Over the follow-up period, the mean number of BCM measurement per patient was 7.4, whilst the mean number of cTnT measurements per patient was 3.4. Demographic details for this cohort are listed in Table 2.

Hydration status correlation with serum troponin

For our cohort of 336 patients, the mean (SEM) hydration status was +1.15 (0.12) L and the median (IQR) cTnT level was 43.5 (20-90) ng/L. The cTnT results were not normally distributed and were therefore transformed logarithmically. There was a statistically significant correlation between Log (cTnT) with the OH value (the Spearman r value was 0.425, $P < 0.0001$, Figure 1).

Association of overhydration with cardiac death

Over a median follow-up period of 23.9 mo, 48 patients (14.3%) of the 336 PD patients died. Cardiac causes of death (sudden cardiac death, cardiac failure or myocardial ischaemia) were assigned in 13 (27%) of cases. The patients that died from cardiac or non-cardiac causes were similar with respect to their age, incidence of diabetes mellitus, gender, ethnicity and cause of renal failure (Table 1). However, the patients with cardiac causes of death had significantly shorter dialysis vintage (10.3 mo *vs* 37.0 mo, $P < 0.0001$) and were significantly more overhydrated by BCM measurement (2.95 L *vs* 1.35 L, $P < 0.05$). The OH status appeared to predict cardiac death that occurred at a mean of 15.5 mo subsequent to the BCM readings. The mean duration between the BCM reading and non-cardiac death was 16.1 mo.

Correlation between dynamic changes in OH and dynamic changes in cTnT over 6 mo

We identified a sub-group of patients that were severely overhydrated; median (IQR) hydration at baseline was +2.7 (2.3 to 3.7) L. They were followed up for a minimum of 6 mo. For each individual, the rate of change of OH (Δ OH) was calculated using the "least squares" method to estimate the straight line that best fitted that patient's

Table 1 Baseline demographic

	All	Survivors	Non-cardiac death	Cardiac death	*P*-value (comparing cardiac *vs* non cardiac death patients)
No.	336	288	35	13	
Age[1]	57.9 (48.1-69.0)	55.4 (46.9-66.6)	68.9 (61.8-77.0)	68.9 (62.9-76.5)	NS
Male	207 (62%)	167 (58%)	27 (77%)	13 (100%)	NS
Diabetes mellitus	123 (37%)	99 (34%)	15 (43%)	9 (69%)	NS
Assessed as suitable for transplantation	159 (47%)	148 (51%)	10 (29%)	1 (8%)	NS
Dialysis vintage (mo)[1]	7.6 (0.9-31.4)	6.5 (0.8-24.0)	37.0 (4.0-57.4)	10.3 (2.9-23.1)	< 0.00001
Body composition measurements: Mean (SEM)					
OH (L)	1.15 (0.12)	1.04 (0.13)	1.35 (0.32)	2.95 (0.78)	< 0.05
Lean tissue index	13.7 (0.5)	13.9 (0.5)	11.9 (0.5)	12.3 (1.6)	< 0.0001
Fat tissue index	13.2 (0.3)	13.3 (0.3)	11.9 (0.6)	13.3 (1.0)	< 0.01
Ethnicity					
Whites	112 (33%)	97 (34%)	14 (40%)	1 (8%)	NS
Blacks	67 (20%)	59 (20%)	5 (14%)	3 (23%)	
Asians	139 (41%)	117 (41%)	15 (43%)	7 (54%)	
Others	18 (5%)				
Cause of renal failure: *n* (%)					
Unknown	82 (24%)	66 (30%)	8 (23%)	2 (15%)	NS
GN	51 (15%)	26 (12%)	1 (3%)	0 (0%)	
Cancer/trauma	1 (0%)	3 (1%)	0 (0%)	0 (0%)	
Congenital/familial	8 (2%)	5 (2%)	0 (0%)	0 (0%)	
Diabetes	105 (31%)	69 (31%)	14 (40%)	9 (69%)	
Hypertension	28 (8%)	16 (7%)	5 (14%)	2 (15%)	
APKD	12 (4%)	11 (5%)	1 (3%)	0 (0%)	
TIN/chronic pyelonephritis	49 (15%)	29 (13%)	6 (17%)	0 (0%)	
Blood results					
Baseline log(CRP) (mg/L)	0.57 (0.03)	0.52 (0.04)	0.79 (0.12)	0.81 (0.20)	NS
Time av log (CRP) (mg/L)	0.67 (0.04)	0.61 (0.04)	1.09 (0.11)	0.98 (0.17)	NS
Baseline albumin (g/L)	38.9 (0.3)	39.3 (0.3)	36.1 (1.0)	36.8 (1.0)	< 0.05
Time av albumin (g/L)	37.6 (0.2)	38.2 (0.2)	33.8 (1.0)	35.6 (1.1)	< 0.002
Baseline haemoglobin (g/dL)	11.0 (0.1)	10.9 (0.1)	11.8 (0.3)	11.0 (0.5)	NS
Time av haemoglobin (g/dL)	11.2 (0.1)	11.2 (0.1)	11.7 (0.2)	10.7 (0.3)	NS

Values represent mean (SEM) or number (%) unless denoted by[1] (values shown are median, interquartile ranges). OH denotes the "overhydration" reading from the body composition monitor. GN: Glomerulonephritis; APKD: Adult polycystic kidney disease; TIN: Tubular-interstitial nephritis; OH: Overhydration.

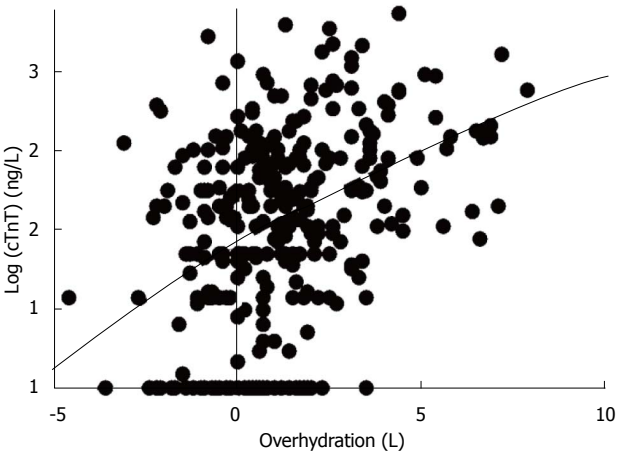

Figure 1 Regression analysis of baseline overhydration *vs* cardiac troponin T levels. cTnT: Cardiac troponin-T.

data. The median (IQR change of OH over 6 mo was -0.7 (-0.3 to -1.5) L. The median (IQR) baseline cTnT value was 60 (37-100) ng/L (Table 2). We plotted the ΔOH with ΔcTnT and found a statistically significant correlation (Spearman *r* value = 0.29, $P < 0.02$; Figure 2). The rates of change of FTM and LTM were also calculated using the "least squares" method. Over a 6-mo period, there was a FTM increase of +2.3 kg (IQR: 0.1-3.7, $P < 0.0001$, Wilcoxon Signed Rank test). By contrast, patients showed a statistically significant loss of LTM during the follow-up, equivalent to -1.5 kg (IQR: -4.5 to -1.7, $P < 0.05$; Table 2). There were no significant correlation between ΔOH and any of the biochemical nutrition or inflammatory markers (either baseline or time-average values of serum albumin, haemoglobin and CRP).

DISCUSSION

In our current study, patients who died from cardiac causes were more severely overhydrated than patients who died from other diseases although this association does not indicate causality or reversibility. This is in keeping with a recent pre-dialysis study that also showed an association between cardiac (as well as all-cause mortality) for patients with chronic kidney disease stages 4 and 5 with fluid overload determined by BIS[14]. We

Table 2 Baseline demographic of cohort that had overhydration > 2 L

Sub-group: Patients with severe OH	
Number	66
Age[1]	60.1 (51.1-71.1)
Male (%)	44 (67%)
Diabetes mellitus (%)	27 (41%)
Assessed as suitable for transplantation (%)	26 (39%)
Dialysis vintage (mo)[1]	1.79 (0.5-32.1)
Body composition measurements:	
Baseline OH (L)[1]	2.7 (2.3-3.7)
OH over 6 mo (L/6 mo)[1]	-0.7 (-0.3--1.5)
Baseline FTM (kg)[1]	23.6 (17.3-28.9)
FTM over 6 mo (kg)[1]	2.3 (0.1-3.7)[3]
Baseline LTM (kg)[1]	37.2 (29.6-44.6)
LTM over 6 mo (kg)[1]	-1.5 (-4.5-1.7)[2]
Number of readings	7.4 (0.4)
Cardiac troponin T measurements (ng/L)	
Baseline cTnT[1]	60 (37-100)
Final cTnT[1]	71 (37-115)
Number of readings	3.4 (0.2)
Ethnicity: *n* (%)	
Whites	26 (39%)
Blacks	14 (21%)
Asians	23 (35%)
Others	3 (5%)
Cause of renal failure: *n* (%)	
Unknown	15 (23%)
GN	9 (14%)
Cancer/trauma	2 (3%)
Congenital/familial	2 (3%)
Diabetes	23 (35%)
Hypertension	6 (9%)
APKD	0 (0%)
TIN/chronic pyelonephritis	9 (14%)
Blood results	
Baseline log (CRP) (mg/L)	0.67 (0.08)[4]
Time average log (CRP) over 6 mo (mg/L)	0.73 (0.08)[4]
Baseline albumin (g/L)	36.4 (0.6)[4]
Time average albumin over 6 mo (g/L)	36.0 (0.6)[4]
Baseline haemoglobin (g/dL)	10.5 (0.2)[4]
Time average haemoglobin over 6 mo (g/dL)	10.6 (0.2)[4]

Values represent mean (SEM) or number (%) unless denoted by[1] (values shown are median, interquartile ranges). [2]$P < 0.05$ by Wilcoxon Signed Rank Test to determine if Median is ≠ 0; [3]$P < 0.0001$ by Wilcoxon Signed Rank Test to determine if Median is ≠ 0; [4]There were no significant correlation with OH by Pearson r correlation. OH denotes the "overhydration", FTM denotes fat tissue mass and LTM denotes lean tissue mass from the body composition monitor. GN: Glomerulonephritis; APKD: Adult polycystic kidney disease; TIN: Tubular-interstitial nephritis; OH: Overhydration.

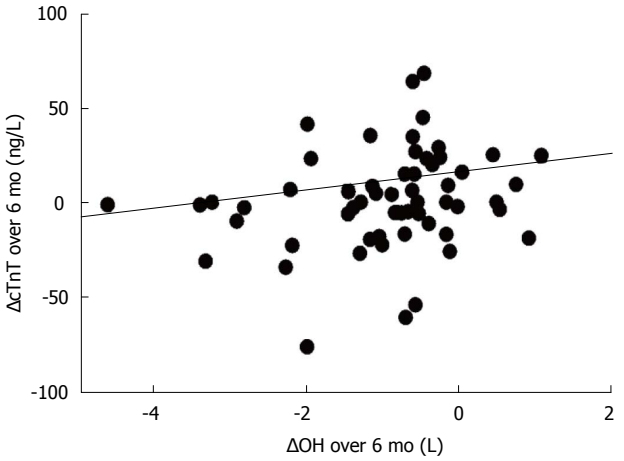

Figure 2 Regression analysis of the change in overhydration *vs* cardiac troponin T levels. OH: Overhydration; cTnT: Cardiac troponin T levels.

also note that a recent report[15] showed that patients randomized to having their hydration status managed with BCM had lower mortality.

Cardia cTnT has been repeatedly shown to be predictive of cardiac death in patients on dialysis[16,17]. It is therefore significant that we found a direct correlation between OH status and Log cTnT ($r = 0.425$, $P < 0.0001$). These results alone do not prove causality and it remains possible that this association is due to PEW/MIA. After all, a large database of haemodialysis patients (MONDO) confirmed that the BIS parameters of FTI and LTI were also associated with mortality[18]. Unfortunately, for a retrospective study, it was difficult to define the exact causes of deaths that were ascribed at the time to be "cardiac/sudden cardiac deaths".

However, uniquely we also found a statistically significant correlation between the improvement in hydration status and decrement in cTnT levels over 6 mo ($r = 0.29$, $P < 0.02$; Figure 2). Patients in this cohort study had a median baseline OH value of +2.7 L and showed a median decrement over 6 mo of -0.7 L. It is unlikely that this magnitude of change is a consequence of correcting malnutrition/PEW. In fact, the BIS data suggested a small but significant loss of LTM over these 6 mo, which may have been due to patients' salt and fluid restriction with consequent diminished dietary protein intake. We also found that there were no correlation between either baseline or time-averaged biochemical markers of nutrition and inflammation suggesting the improvement in cTnT is likely to be a consequence of fluid status and not nutrition. Of course, it must be acknowledged that using albumin, CRP and presence of anaemia to be indicators of nutrition is imprecise. Nevertheless, we found no signal to suggest changes in nutrition status was a cofounder for the change in cTnT.

It is important to note that our cohort exhibited an increase in FTM that was equivalent to +2.3 kg (IQR: 0.1-3.7) over 6 mo. It is possible that the fat gain was exacerbated through increased use of hypertonic glucose dialysate to improve hydration status.

Although our results do not prove that correction of overhydration will lead to reduced cardiac mortality, it supports the finding by Onofriescu *et al*[15]. We hope these findings provide added impetus for clinicians to focus on OH particularly as we have previously reported that BCM guided reduction of OH does not cause excessive loss of residual renal function[19,20]. We note that improved hydration status in this study appeared to come at the expense of increasing FTM but the clinical impact of increasing obesity is unclear. In the normal population, obesity is associated with glucose intolerance and cardiovascular risk but in HD, "reverse epidemiology" has been reported (HD patients with high BMI have a survival

advantage)[21]. Similarly, studies have shown that PD patients with high FTM have a survival advantage[18,22].

Limitations of this study include the retrospective nature of the data collection and the fact that we included both incident and prevalent patients. BCM measurements were performed with dialysate *in situ* and this may have reduced the precision of the measurements[23] (although we were consistent in how we performed the measurements). Most critically, our retrospective study can only demonstrate associations and cannot determine causality. The study would have also benefited if we had a formal assessment of cardiac function. Future studies could include simple assessments such as echocardiography but unfortunately contemporaneous echo studies with bioimpedance measurements were not available for all subjects in our study. Equally, it was difficult in a retrospective study to accurately determine "cause" of fluid overload.

In conclusion, we believe our data provides indirect evidence to suggest that overhydration impacts negatively on the heart. Intriguingly, our data also suggests that correcting overhydration is possible and may lead to improved cardiac prognosis but perhaps at the expense of increasing obesity. Randomized controlled studies on this subject will be difficult, but it will be interesting to await the results of two studies that are designed to explore the impact BIA might have on left ventricular mass[24] and survival[25,26].

COMMENTS

Background

Despite the importance of euvolaemia, there is still much debate on the most clinically useful method of volume assessment. Overhydration (OH) as measured by body composition monitor (BCM) is associated with increased mortality in dialysis patients. BCM has been used to guide treatment on the assumption that correcting OH will improve cardiac morbidity and mortality, although data demonstrating causality that is reversible is limited.

Research frontiers

It has often been assumed that the observed relationship between OH and mortality is related to cardiovascular damage. However, an important caveat is the ratio of extracellular water to total body water is also increased in the setting of muscle wastage. Thus it is possible that the increased mortality associated with OH relate to its association with protein energy wasting (PEW)/malnutrition inflammation atherosclerosis (MIA) Syndrome.

Innovations and breakthroughs

Most research into this area has focused on single time point measurements of OH and cardiac biomarkers. While cardiac troponins-T (cTnT) have been repeatedly shown to be predictive of cardiac death in dialysis patients, the effect of malnutrition on the observed relationship between OH, cardiac biomarkers and outcomes is difficult to establish. More recent trials have shown that targeting a reduction in OH is associated with better survival. However, the temporal relationship of cardiac biomarkers and reduction in OH has not been well described.

Applications

In this study, peritoneal dialysis patients who died of cardiac causes had higher OH, compared to patients that died from other causes. Over a 6-mo period, the authors found that reducing OH in severely overhydrated patients was associated with corresponding decrements in cTnT. There was no significant correlation between change in OH and any of the biochemical or nutritional markers studied, suggesting that the improvement in cTnT is likely to be a consequence of fluid status and not nutrition. Although the results do not prove that correction of OH will lead to reduced cardiac mortality, the temporal association observed between OH and cTnT supports the role of fluid status in cardiac risk management of dialysis patients.

Terminology

OH is a mathematically derived estimate of excess fluid. The BCM expresses the body weight in terms of lean tissue mass (LTM-mainly muscle), adipose tissue mass (ATM-mainly fat) and OH. Each of these compartments has a specific composition and contains a known quantity of water per mass of tissue. The water of LTM and ATM consist of differing proportion of extracellular and intracellular water in addition to solid components. Excess fluid represents an expansion of only the extracellular water, whereas ICW remains unchanged. The excess fluid may reside within adipose tissue or lean tissue raising the hydration of the respective tissue above the "normal" values (*e.g.,* oedema). Alternatively, excess fluid may simply appear as a distinct compartment without altering the hydration of the major tissues (*e.g.,* ascites, pleural effusion). As the extracellular hydration of LTM and ATM is known, the expected "normal" volume of ECW of these tissues can be calculated. The difference between "normal" ECW and measured ECW is the excess fluid, OH.

Peer-review

OH is gaining popularity as an objective measurement of fluid status due to its relatively low cost and ease of measurement. There have been many studies on this topic, but it is uncommon to find studies on repeated measurements of OH and cardiac biomarkers. This study provides indirect evidence to suggest that OH is associated with worse cardiac outcomes, and importantly, that correcting OH may lead to improved cardiac prognosis.

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ORIGINAL ARTICLE

Observational Study

Factors associated with regular dental visits among hemodialysis patients

Masami Yoshioka, Yasuhiko Shirayama, Issei Imoto, Daisuke Hinode, Shizuko Yanagisawa, Yuko Takeuchi, Takashi Bando, Narushi Yokota

Masami Yoshioka, Shizuko Yanagisawa, Department of Oral Health Science and Social Welfare, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 7708504, Japan

Yasuhiko Shirayama, Yuko Takeuchi, Department of Community Medicine and Welfare, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 7708504, Japan

Issei Imoto, Department of Human Genetics, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 7708503, Japan

Daisuke Hinode, Department of Hygiene and Oral Health Science, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 7708504, Japan

Takashi Bando, Department of Oral Surgery, Kawashima Hospital, Tokushima 7700011, Japan

Narushi Yokota, Department of Kidney Disease (Artificial Kidney and Kidney Transplantation), Kawashima Hospital, Tokushima 7700011, Japan

Author contributions: Yoshioka M was the principal investigator and was involved in study conception, design and supervision; Shirayama Y participated in study design; Imoto I participated in study design and critically reviewed the manuscript; Hinode D participated in statistical analysis and critically reviewed the manuscript; Yanagisawa S performed data collection and input; Takeuchi Y performed data collection and input; Bando T and Yokota N arranged the interviews at the dialysis facilities; all authors have given their approval for the final version of the paper to be published.

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Correspondence to: Masami Yoshioka, DDS, PhD, Associate Professor, Department of Oral Health Science and Social Welfare, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramotocho, Tokushima 7708504, Japan. yoshioka.masami@tokushima-u.ac.jp
Telephone: +81-88-6339171
Fax: +81-88-6339171

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Abstract

AIM

To investigate awareness and attitudes about preventive dental visits among dialysis patients; to clarify the

barriers to visiting the dentist.

METHODS

Subjects included 141 dentate outpatients receiving hemodialysis treatment at two facilities, one with a dental department and the other without a dental department. We used a structured questionnaire to interview participants about their awareness of oral health management issues for dialysis patients, perceived oral symptoms and attitudes about dental visits. Bivariate analysis using the χ^2 test was conducted to determine associations between study variables and regular dental check-ups. Binominal logistic regression analysis was used to determine factors associated with regular dental check-ups.

RESULTS

There were no significant differences in patient demographics between the two participating facilities, including attitudes about dental visits. Therefore, we included all patients in the following analyses. Few patients (4.3%) had been referred to a dentist by a medical doctor or nurse. Although 80.9% of subjects had a primary dentist, only 34.0% of subjects received regular dental check-ups. The most common reasons cited for not seeking dental care were that visits are burdensome and a lack of perceived need. Patients with gum swelling or bleeding were much more likely to be in the group of those not receiving routine dental check-ups (χ^2 test, $P < 0.01$). Logistic regression analysis demonstrated that receiving dental check-ups was associated with awareness that oral health management is more important for dialysis patients than for others and with having a primary dentist ($P < 0.05$).

CONCLUSION

Dialysis patients should be educated about the importance of preventive dental care. Medical providers are expected to participate in promoting dental visits among dialysis patients.

Key words: Hemodialysis; Questionnaire; Oral health; Dental visit; Health management

Core tip: We investigated dialysis patients' awareness and attitudes about preventive dental visits, and tried to clarify the barriers to visiting the dentist. Subjects included 141 dentate outpatients receiving hemodialysis treatment. We interviewed participants using a structured questionnaire. The common reasons dialysis patients cited for not seeking dental care were lack of concern and/or lack of awareness of the importance of preventive dental visits. Medical practitioners rarely refer dialysis patients for dental care. Our findings suggest that dialysis patients should be educated about the importance of preventive dental care. Medical providers are expected to participate in promoting dental visits among dialysis patients.

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INTRODUCTION

As of December 31, 2013, there were 314180 dialysis patients in Japan, a number that has been increasing yearly[1]. Most dialysis facilities do not have dental departments[2]. Prior to this study, we hypothesized that inconvenient accessibility could be a barrier to dental visits among dialysis patients. Dialysis patients have a high risk of dental caries and periodontitis[3,4]. Recently, several studies have reported a significant association between moderate to severe periodontitis and mortality among hemodialysis patients[5-7]. Therefore, preventive dental care should be considered very important for this population.

In this study, we tried to determine factors associated with regular dental visits and to determine barriers to preventive dental care among hemodialysis patients, to promote improvement in holistic oral health management.

MATERIALS AND METHODS

Outpatients receiving hemodialysis treatment at two dialysis facilities, one with a dental department (Facility A) and the other without (Facility B), were included in this study. The total number of patients receiving hemodialysis at Facility A was approximately 600; approximately 150 received hemodialysis at Facility B. The inclusion criteria for this study were outpatients receiving hemodialysis treatment three times per week, and who agreed to participate in the study. Because we needed to interview patients at their bedsides, we excluded patients who had difficulty conversing independently. We used a structured questionnaire to interview 141 dentate patients about their awareness of oral health management issues, their perceived oral symptoms and their attitudes about seeking dental care (Figure 1). Written informed consent for participation was not obtained from the participants in this study; we regarded replying to the interview questions as signifying agreement to participate, as we explained in the document that was provided to each patient at the start of the interview.

Statistical analyses were performed with the SPSS 17.00 statistical package (SPSS Japan Inc., Tokyo, Japan). Bivariate analysis using the χ^2 test was conducted to determine associations between study variables and regular dental check-ups. Binominal logistic regression analysis was used to determine factors associated with regular dental check-ups. Statistical significance was accepted at a level of 0.05

Questionnaire
Age sex (male/female)
How long have you been receiving dialysis treatment? Years months
Are you employed? (yes/no)
Is there a dental department at the facility where you receive dialysis treatment? (Yes/No/Not sure)
Have you ever been referred to a dentist by a medical practitioner? (Yes/No)
Do you think that oral health management is more important for patients receiving dialysis treatment than for others? (Yes/No) If "Yes," why do you think so?
How many teeth do you have? [Most (≥ 20) / half (10-19) / few (< 10) / zero]
Do you have dentures? (Yes/no/unused)
Do you have any oral symptoms? (1) toothache/sensitivity; (2) loose tooth; (3) gum swelling/bleeding; (4) food impaction; (5) bad breath; (6) sticky mouth; (7) crooked teeth; (8) malocclusion; (9) clicking of the jaw; (10) missing tooth; (11) dry mouth; (12) rough lips; (13) abnormal taste; (14) frequent stomatitis; (15) odd feeling to dentures; (16) other
Do you have a primary dentist? (Yes/no)
Do you receive a dental check-up once a year or more? (Yes/no)
When did you last visit a dentist? Years, months ago
When do you visit the dentist? (1) I visit regularly, even without a specific problem. (2) I visit irregularly, when I have a specific problem. (3) I occasionally do not visit the dentist, even if problems are present. (4) I never visit the dentist.
If you answered, "I do not receive dental check-ups," why? (1) no perceived need; (2) it is burdensome; (3) lack of time; (4) anxiety about dental treatment; (5) physical barrier (fatigue); (6) psychological barrier (fear/pain/hate); (7) economic burden; (8) lack of accessibility; (9) no attendant; (10) no reliable dentist; (11) other
Please suggest ideas that would make it easier for dialysis patients to receive dental check-ups.

Figure 1 Questionnaire.

and lower.

RESULTS

Distribution of participants

The distribution of respondents is shown in Table 1. The age of the respondents ranged from 29 to 86 years, with a mean age of 63.1 years (SD 11.0). The mean duration of dialysis was 10.3 years (SD 8.7); 42.6% had been receiving dialysis for more than 10 years. The percentage of employed patients was 34.8%.

There were no significant differences in patient demographics between the two participating facilities, including attitudes about dental visits. Therefore, we included all patients in the following analyses.

Awareness of oral health management issues

Only 4.3% of subjects had been referred to a dentist by their medical practitioner. Twenty-three percent of the respondents considered oral health management to be important for dialysis patients; most of these were aware of the association between periodontitis and general health conditions.

Self-reported oral health status

Self-reported oral health conditions are shown in Table 2. Oral health problems reported by dialysis patients included dry mouth (39.0%), bad breath (34.8%) and gum swelling/bleeding (20.6%).

Factors associated with dental visits

Eighty percent of subjects had a primary dentist, but only 34% of participants received regular dental check-ups. However, 66.0% of subjects had visited a dentist in the past year, suggesting that a considerable number of oral problems had arisen. As for the timing of dental visits, 56.0% of subjects answered that they visited a dental office only when symptoms arose; 5.7% answered that they sometimes refused to visit a dentist even if oral symptoms were present. The reasons cited for not seeking a dental check-up are shown in Table 3. The most common reasons given were "it is burdensome" and "no perceived need", followed by "lack of time" and "psychological barrier (fear/pain/hate)". As shown in Table 4, χ^2 testing demonstrated that receiving regular dental check-ups was significantly associated with awareness of oral health management issues related to dialysis and with having a primary dentist ($P < 0.01$). The prevalence of self-reported gum swelling/bleeding was higher among those not receiving dental check-ups than among those receiving dental check-ups ($P < 0.01$). Binominal logistic regression analysis using "receiving dental check-ups" as the outcome variable demonstrated that receiving dental check-ups was significantly associated with awareness of oral health management issues related to dialysis treatment, having many teeth, having dentures and having a primary dentist (Table 5).

DISCUSSION

Because the interviewer in this study was from a third party, not from a dialysis facility or a private dental clinic, and because personal information was completely anonymized, we believe that we were able to elicit patients' opinions and thoughts without bias. Barriers to visiting the dentist included a lack of awareness of the need for care, cost and fear of dental procedures[8,9]. Especially among patients with special health care needs, dental fear and/or anxiety is considered the most common barrier to accessing oral health care[10]. Prior to this study, we had hypothesized that time restrictions or general fatigue would be the main reasons that dialysis

Table 1 Demographic profiles of participants

Facility	Facility A With dental department	Facility B Without dental department	Total
Number of subjects	88	53	141
Age	61.9 ± 11.6	65.1 ± 9.7	63.1 ± 11.0
Sex			
Male	59	31	90
Female	29	22	51
Duration of dialysis			
< 1 yr	6	4	10
1-4 yr	23	12	35
5-9 yr	21	15	36
⩾ 10 yr	38	22	60
Employment			
Employed	35	15	50
Unemployed	53	38	91
Primary dentist			
Yes	68	46	114
No	20	7	27
Dental check-up			
Yes	31	17	48
No	57	36	93

Table 2 Self-reported oral health status (*n* = 141)

	No.	% of Subjects
Number of teeth		
⩾ 20	101	71.6
10-19	25	17.7
1-9	15	10.6
Possession of denture		
Yes	34	24.1
No/unused	107	75.9
Oral symptom		
Toothache/sensitive	26	18.4
Shaking tooth	22	15.6
Gum swelling/bleeding	29	20.6
Food impaction	104	73.8
Bad breath	49	34.8
Sticky mouth	30	21.3
Crooked teeth	21	14.9
Malocclusion	28	19.9
Clicking of jaw joint	14	9.9
Lack of tooth	13	9.2
Dry mouth	55	39.0
Rough lip	22	15.6
Wrong taste	15	10.6
Frequent stomatitis	20	14.2
Odd feeling to denture	2	1.4
Other	8	5.7

patients do not seek dental care. As shown in Table 3, some patients answered "no time to go" as a reason for not seeking dental care. However, we found that lack of concern and/or lack of awareness of the need for preventive dental visits were common reasons in this population. In Japan, most dental care is covered by medical insurance. In fact, dialysis patients are sometimes provided with additional insurance benefits. Therefore, nobody answered "economic burden" as a reason for not seeking dental care.

Table 3 Reasons for not seeking dental care (*n* = 93)

	No.	% of subjects
No perceived need	33	23.4
Burdensome	36	25.5
No time to go	16	11.3
Anxiety for dental treatment	2	1.4
Physical burden (fatigue/tired)	3	2.1
Psychological burden (fear/painful/hate)	13	9.2
Economic burden	0	0.0
Uneasy accessibility	0	0.0
No attendant	2	1.4
No reliable dentist	0	0.0
Others	10	7.1

Recently, the close relationship between periodontal disease and systemic disease has been highlighted[11,12]. It has been reported that severe periodontitis can affect mortality in hemodialysis patients[5-7]. Studies involving patients with chronic kidney disease found that efficient initial periodontal therapy lowered serum levels of some inflammatory biomarkers[13,14].

Our results showed that awareness of the oral health management issues of dialysis patients led to preventive dental visits in this population. Therefore, providing dialysis patients with information about the relationship between periodontitis and systemic conditions might effectively promote preventive oral health care.

Dialysis patients tend to be at high risk for tooth decay and periodontal disease[15]. Oral surgical procedures require extra precautions in these patients because of associated medications (*e.g.,* anticoagulants) and complications (*e.g.,* hypertension, diabetes). Therefore, dialysis patients must be informed of their greater need for preventive dental care compared with the general population.

Medical history and/or drug use can impact oral health; however, we did not investigate those parameters and therefore cannot draw conclusions on that subject. However, we found that patients with gum swelling or bleeding were much more likely to be in the group of those not receiving routine dental check-ups. This finding suggests that gingival inflammation caused by other illnesses and/or drug use might not lead to routine dental visits.

The percentage of subjects receiving regular dental checkups was 34.0% in this study. According to the National Health and Nutrition Survey of 2012, 47.8% of adults and 55.3% of individuals in their sixties had received a dental check-up in the past year[16]. A survey in 2010 in Tokushima, the same prefecture in which the present study was carried out, reported those percentages to be 43.6% and 51.0%, respectively[17]. Therefore, the percentage of dialysis patients who sought dental checkups in this study was lower than that of the general population.

In a previous study, we showed that most hemodialysis outpatients in Japan received dialysis treatment at a facility without a dental department[2]. The present

Table 4 Distribution of subjects receiving *vs* not receiving dental checkups, according to study variable (χ^2 test)

Variable	Receive dental check-up		Not receive dental check-up		*P*
	n[1]	%[2]	*n*[1]	%[2]	
Sex					
Male	29	60.4	61	65.6	0.545
Female	19	39.6	32	34.4	
Employment					
Employed	15	31.3	34	36.6	0.502
Unemployed	33	68.8	59	63.4	
Referral to dental visit by medical practitioner					
Yes	4	8.3	2	2.2	0.102
No	44	91.7	91	97.8	
Possession of denture					
Yes	17	35.4	17	18.3	0.024
No/unused	31	64.6	66	71.0	
Gum swelling/bleeding					
Yes	4	8.3	25	26.9	0.007
No	44	91.7	68	73.1	
Consciousness of oral health management because of dialysis treatment					
Yes	18	37.5	15	16.1	0.005
No	30	62.5	78	83.9	
Having a primary dentist					
Yes	46	95.8	68	73.1	0.001
No	2	4.2	25	26.9	

[1]*n*: Total number of subjects corresponding to each answer; [2]%: The percentage of subjects who answered "receive a dental check-up" or "not receive a dental check-up".

Table 5 Factors associated with receiving dental check-ups, according to binominal logistic regression analysis[1] (*n* = 141)

Variable	OR	95%CI	*P*-value
Consciousness of oral health management because of dialysis treatment	3.241	1.298-8.125	0.012
Number of teeth	2.361	1.060-5.258	0.035
Possession of denture	4.209	1.271-13.933	0.019
Having a primary dentist	6.138	1.279-29.456	0.023
Gum swelling/bleeding	5.831	1.659-20.499	0.006

[1]Binominal logistic regression analysis was conducted using each of five variables as the dependent variable.

study included dialysis patients at facilities with and without dental departments. We found no difference between the facilities in the percentage of patients receiving dental check-ups. Few patients at either facility had been referred for a dental visit by their medical practitioner. Education on the importance of regular dental care is necessary for dialysis patients. Moreover, medical providers are expected to participate in promoting dental visits among dialysis patients.

In conclusion, recognition that oral health management is more important for dialysis patients than for the general population might increase regular dental visits in this population. We found that patients who received dental check-ups had fewer symptoms of gum swelling or bleeding, suggesting that periodic dental visits could be effective in preventing an inflammatory response. Medical providers should participate in promoting dental visits among dialysis patients.

COMMENTS

Background

In Japan, a number of dialysis patients have been increasing yearly. Since dialysis patients have a high risk of dental caries and periodontitis, preventive dental care should be considered very important for this population. In this study, they tried to determine factors associated with dental visits and to determine barriers to preventive dental care among hemodialysis patients.

Research frontiers

Recently, several studies have reported that severe periodontitis can affect mortality in hemodialysis patients. Studies involving patients with chronic kidney disease (CKD) found that efficient initial periodontal therapy lowered serum levels of some inflammatory biomarkers in CKD patients. Therefore, oral health management towards dialysis patients gets attention. The research hotspot is to elucidate the factors associated with dental visits among hemodialysis patients in order to resolve the barriers for dental visits.

Innovations and breakthroughs

Recently, the close relationship between periodontal disease and systemic disease has been highlighted. Many studies describe the oral health conditions of hemodialysis patients. However, there are very few English language literatures sources concerning preventive dental visit among dialysis patients. The present study elucidated the barriers to visiting the dentist, which the authors must manage with first in order to promote a preventive dental care among dialysis patients.

Applications

The data in this study suggested that awareness of oral health management issues should be strengthen among not only dialysis patients but also medical providers. Furthermore, this study suggested that periodic dental visits could be effective in preventing an inflammatory response.

Terminology

"Preventive dental visits" means that patients visit dental clinic periodically without a specific problem. The purpose of preventive dental visit is often oral examination and professional mechanical tooth cleaning to maintain the favorable oral health condition. "Primary dentist" should offer preventive dental care to their patients in Japan, however, many patients only visit their primary dentist when they have a specific problem in their mouth.

Peer-review

Factors associated with regular dental visits among hemodialysis patients is an absorbing manuscript; the research design is well established and fulfills all the requirements for a clinical study. Besides, the conclusion emphasizes the importance of a multidisciplinary approach to hemodialysis patients attain healthy oral conditions.

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Observational Study

Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature

Geetika Singh, Lavleen Singh, Ranajoy Ghosh, Devajit Nath, Amit Kumar Dinda

Geetika Singh, Lavleen Singh, Ranajoy Ghosh, Devajit Nath, Amit Kumar Dinda, Department of Pathology, All India Institute of Medical Sciences, New Delhi 110029, India

Author contributions: Singh G conceptualized the study, standardized the technique, wrote and critically reviewed the manuscript; Singh L compiled data and wrote the manuscript under guidance of Dr. Geetika Singh; Ghosh R and Nath D helped in standardization of technique; Dinda AK critically reviewed and edited the manuscript.

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Correspondence to: Geetika Singh, MD, Assistant Professor of Pathology, Dr BRAIRCH, Department of Pathology, All India Institute of Medical Sciences, Room No. 1084, First Floor, Teaching Block, New Delhi 110029, India. ggmed22@gmail.com
Telephone: +91-99-71911459
Fax: +91-011-26588663

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Abstract

AIM

To describe the technique of immunofluorescence on paraffin embedded tissue sections and discuss the potential pitfalls with an in depth review of literature.

METHODS

Immunofluorescence is integral to diagnostic renal pathology. Immunofluorescence on paraffin embedded renal biopsies (IF-P) after enzyme treatment has been described in literature, however has not found widespread use in renal pathology laboratories. In our laboratory proteinase K digestion of paraffin embedded renal biopsy material was standardized and applied prospectively in cases where immunofluorescence on fresh frozen tissue was non contributory or not possible. Diagnostic utility was assessed and in a cohort of cases comparison of intensity of staining with routine immunofluorescence was performed.

RESULTS

Over the 5-year study period, of the 3141 renal biopsies received IF-P was performed on 246 cases (7.7%) and was interpretable with optimal digestion in 214 cases (6.8%). It was of diagnostic utility in the majority of cases, which predominantly included glomerular disease. Non-diagnostic IF-P was found in membranous nephropathy (2 of 11 cases), membranoproliferative glomerulonephritis (2 of 32 cases), lupus nephritis (1 of 25 cases), post infectious glomerulonephritis (1 of 11 cases) and chronic glomerulonephritis (3 of 8 cases). Comparing cases with both routine IF and IF-P, 35 of 37 showed either equal intensity or a minor difference in intensity of staining

(1+) for the diagnostic immunoglobulin/complement. Technically assessment of immunofluorescence on the paraffin embedded tissue was found to be easier with clearly observed morphology, however a false positive staining pattern was observed in under-digested tissue.

CONCLUSION

As a "salvage" technique, immunofluorescence on paraffin embedded renal biopsies is of great diagnostic utility, however not without pitfalls.

Key words: Immunofluorescence on paraffin section; Renal biopsy; Salvage technique; Enzymatic digestion; Proteinase K

Core tip: Immunofluorescence on formalin fixed paraffin embedded tissue is a useful "salvage" technique for renal diagnostic pathology, in case of non-availability of representative fresh frozen tissue. This article describes the technique of immunofluorescence on paraffin embedded tissue sections, discusses the potential pitfalls with an in depth review of literature.

Singh G, Singh L, Ghosh R, Nath D, Dinda AK. Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature. *World J Nephrol* 2016; 5(5): 461-470 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i5/461.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i5.461

INTRODUCTION

Immunofluorescence (IF) is an indispensible technique for rendering an accurate diagnosis in renal pathology. Diseases such as IgA nephropathy (IgAN), C1q nephropathy (C1qN) and C3 glomerulopathy (C3G) cannot be diagnosed without IF. Direct immunofluorescence (DIF) on fresh frozen tissue (IF-F) is the most widely used IF technique. Not uncommonly, however IF-F is not satisfactory due to non-representative sampling (medulla) or is not possible due to unavailability of fresh unfixed tissue, such as in referral cases and archived tissue. This leads to incomplete diagnosis and suboptimal patient management. To overcome these hurdles a method of enzymatic digestion of formalin fixed paraffin embedded tissue was standardized and introduced in our laboratory in 2011.

Enzymatic digestion breaks the protein cross linkages formed during formalin fixation[1] thereby exposing the antigenic immune complexes to staining with FITC (fluorescein isothiocyanate) labeled antibodies. Though this technique has been described in literature using different enzymes with the earliest report in 1976[2], it is still not in widespread use in laboratories handling renal biopsies.

We discuss our experience with this technique in day-to-day diagnostic renal pathology, its utility in reaching final diagnoses and compare it with usual IF-F where available. Technical and interpretation issues faced are described in detail, and may be helpful to any laboratory planning to introduce this technique.

MATERIALS AND METHODS

Standardization: In a case of diffuse proliferative lupus nephritis, proteinase K (Sigma Aldrich, United States) enzymatic digestion was standardized (at concentrations according to manufacturer's protocol) with variation in timing of exposure at room temperature. Results were compared for the adequacy of digestion and intensity of staining for FITC-IgG.

Selection of cases

IF-P was performed prospectively in cases where there was inadequate/non representative fresh frozen tissue, in referral blocks where fresh frozen tissue was not available and in cases where the renal pathologists wanted to confirm the findings of routine IF-F. The FITC labeled antibodies to be applied were dictated by light microscopic differential diagnoses in the case and included both full panel (IgA, IgG, IgM, C3, C1q, kappa and lambda) as well as limited panels.

Interpretation of immunofluorescence

In cases where there was optimal digestion and adequate material the IF-P results were evaluated by 2 renal pathologists (LS and GS) and semiquantitatively graded on a 0-3+ scale. In cases where IF-F was available for comparison, these were graded independently in a blinded manner and compared to the grading of IF-P results. All immunofluorescence images were digitally captured and archived.

RESULTS

Enzyme digestion with proteinase K was standardized and the protocol followed is described in Table 1. Standardization was performed at room temperature and slight variations in enzyme exposure depending on ambient temperature (ranging from 15 to 20 min) gave optimal digestion results. This obviated the need for maintaining slides at 37 ℃ in a water bath.

In the 5-year study period between March 2011 and May 2015, 3171 biopsies (both native and transplant) were received. IF-P was performed on a total of 246 cases (7.7%). The results could not be interpreted in 32 cases (13%) due to technical issues of under digestion (18 cases) and floating of tissue/inadequate tissue (14 cases).

Therefore in 214 cases with adequate tissue, optimal digestion was achieved. Optimal digestion was determined on each individual slide by observing the

Table 1 Protocol for immunofluorescence on paraffin embedded renal biopsies

Cut formalin fixed paraffin embedded tissue at 3-4 μ thickness on poly-L-Lysine coated slides
Deparrafinize and rehydrate tissue sections
Immerse in Tris EDTA pH 9 for 30 min at room temperature
Perform enzymatic digestion with proteinase K 1.25 mg/mL (Sigma Aldrich, United States) at room temperature for 15 min[1]
Stop digestion by immersing in Tris EDTA at 4 ℃
Leave in Tris EDTA for 40 min at 4 ℃
Rinse in PBS for 10 min
Apply FITC conjugated polyclonal rabbit antibodies directed against IgG (dilution 1:50), IgM (1:60), IgA (1:60), C3 (1:30), C1q (1:30), kappa (1:25), and lambda (1:40) (BIOSB, Santa Barbara, CA, United States). Incubate for 2 h in a moist chamber in the dark
Rinse with PBS
Mount in glycerine
Examine slides under a dark field immunofluorescence microscope

[1]Varied based on room temperature. PBS: Phosphate buffered saline; FITC: Fluorescein isothiocyanate.

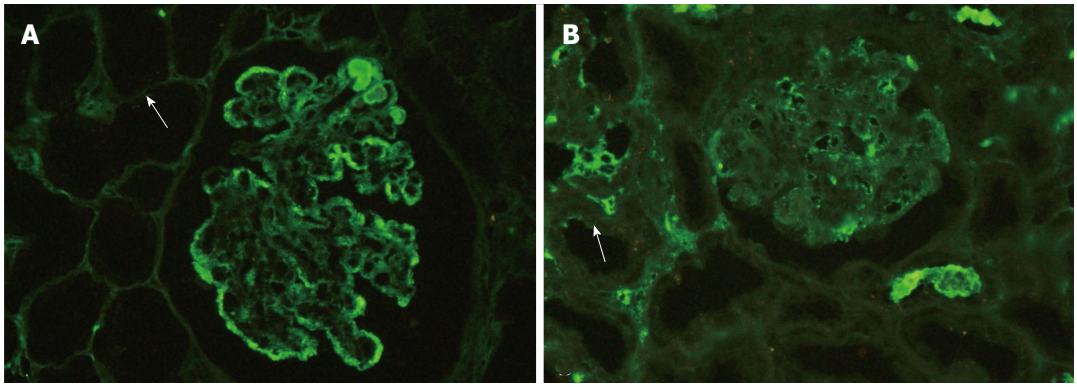

Figure 1 Examples of technically adequate and inadequate digestion. A: Immunofluorescence staining on a paraffin embedded tissue section in a case of diffuse proliferative lupus nephritis after enzymatic digestion with proteinase K. Note the adequate digestion evidenced by disappearance of tubular epithelial cells (arrow) (FITC IgG, × 200); B: Immunofluorescence staining in a case with inadequate digestion with visible tubular epithelial cells (arrow). Note the antibody sticking to the surface of the capillary wall (FITC IgG, × 200). FITC: Fluorescein isothiocyanate.

tubules. From experience, the disappearance of tubular epithelial cell outline with only visible tubular basement membranes correlated with optimal digestion and detection of immune complexes in the tissue (Figure 1A). In under-digested glomeruli, a non-specific staining pattern was observed (Figure 1B) with the antibody appearing to stick to the surface of the capillary walls in a blotchy manner rather than labeling immune complexes/complement with granularity. This staining pattern was recognized as false positive.

The major utility of this technique was in classifying glomerular diseases, with limited utility in tubulointerstitial diseases. Table 2 demonstrates the range of renal pathologies that were diagnosed.

In membranoproliferative glomerulonephritis (MPGN, 32 cases), 30 cases could be adequately diagnosed and sub-classified based on the results of IF-P into immune complex mediated (18 cases) and complement mediated (12 cases) MPGN. In two cases with intramembranous dense transformation of the glomerular basement membrane on electron microscopy (dense deposit disease) no significant C3 deposition was noted on IF-P. One of these cases had a comparative IF-F with the diagnostic C3 dominant pattern and intensity 2+ (0-3+ scale) (Figure 2). One case of IC-MPGN showed 1+ IgG, kappa and lambda with 2+ C3 deposition, but characteristic MPGN type 1 pattern on ultrastructure examination; no comparative IF-F was available. The rest of the cases of MPGN showed at least 2+ intensity of diagnostic immunoglobulin/complement.

Diffuse proliferative glomerulonephritis (12 cases) were diagnosed as post infectious glomerulonephritis (PIGN) in 10 cases based on classical lumpy bumpy deposits of C3 and IgG. In one case with diffuse proliferative exudative pattern of injury and prior episode of febrile illness, a limited IF-P panel of IgA, IgG and C3 was applied to differentiate a PIGN from a proliferative IgA nephropathy. The intensity of IgG and C3 was only 1+, while IgA was negative. The IF-P results were deemed noncontributory in this case. Tissue for electron microscopy was not available.

Within the lupus nephritides (LN, 25 cases) localization of the deposits as mesangial and/or capillary wall aided in accurate classification of the glomerulonephritis (Table 2, Figure 3). The lack of deposits was also significant, as demonstrated by two cases of systemic lupus erythematosus (SLE) presenting with proteinuria and nonspecific light microscopic findings. Further electron microscopic examination confirmed a lupus podocytopathy. In one case of class Ⅱ LN significant

Table 2 Renal pathologies diagnosed by immunofluorescence on paraffin embedded biopsies

Diagnosis	Total number of cases	Number of cases with non diagnostic IF-P (%)	Remarks
MPGN	32	2[1] (6.2%)	Classification into immune complex mediated MPGN (18 cases) and complement mediated MPGN (12 cases) was possible [1]In two cases C3 was not demonstrated and electron microscopy showed features of dense deposit disease
Membranous nephropathy	11	2[1] (22.2%)	In one case staining intensity of IgG was only 1+, however staining pattern was classical [1]In 2 cases significant fine granular immunofluorescence was not noted
Lupus nephritis	25	1[1] (4%)	Classification into Class Ⅱ (2 cases), Class Ⅲ/Ⅳ (15 cases) and Class Ⅴ (5 cases) was possible Two cases of lupus podocytopathy were diagnosed [1]In one case of lupus nephritis only IgM was demonstrated significantly, though electron dense deposits were noted on electron microscopy
Diffuse proliferative glomerulonephritis - post infectious glomerulonephritis	12	1[1] (8.3%)	[1]In one case only 1+ IgG and trace C3 deposition noted No tissue for electron microscopy was available
Pauciimmunecrescentic glomerulonephritis	7	-	-
IgAN	39	-	In 64 cases (minimal change morphology, mesangial proliferation or FSGS), IgAN was excluded by IF-P In one case of diabetic nephropathy IF-P was used to exclude secondary IgAN
C1q nephropathy	2	-	-
Light chain deposition disease	1	-	Tubular basement membrane and vascular deposits were also noted in addition to the glomerular deposits
Amyloidosis	4	-	2 cases of AL amyloid (demonstrating light chain restriction) and 2 cases of AA amyloid
CGN	8	3 (37.5%)	The immune complexes could not be demonstrated in 3 cases of chronic glomerulonephritis, one of these was a case of biopsy proven MPGN and the other was a case of IgAN. In one case of CGN no immune complexes were seen, however no previous renal biopsy record was available
Cast nephropathy	2	-	One case also demonstrated light chain restriction
Tubulointerstitial nephritis	9	-	Associated immune complex mediated glomerular disease was excluded

[1]Non diagnostic cases and details of their immunofluorescence pattern. MPGN: Membranoproliferative glomerulonephritis; CGN: Chronic glomerulonephritis; IgAN: IgA nephropathy; FSGS: Focal segmental glomerulosclerosis.

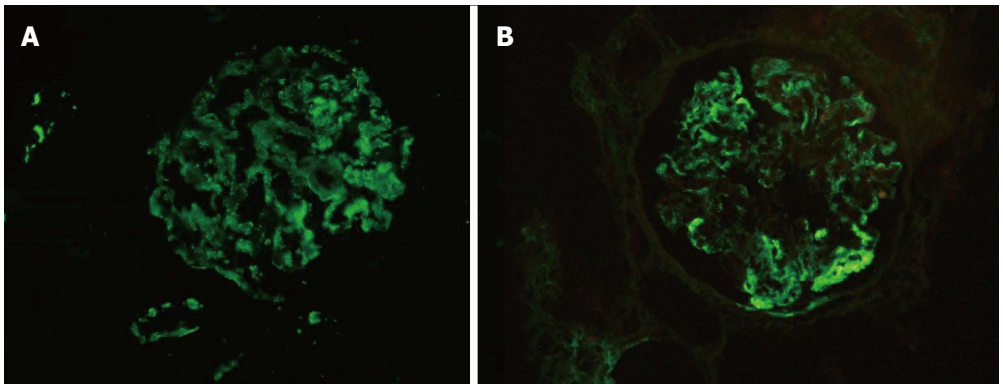

Figure 2 Comparable staining pattern on immunofluorescence on frozen and paraffin embedded tissue. A case of dense deposit disease showing bright C3c deposition 3+ (0-3+ scale) on IF-F (A, FITC C3c, × 200). Note the comparative coarse granular capillary wall staining of C3c (3+) on paraffin embedded tissue section after enzymatic retrieval (B, FITC C3c, × 200). FITC: Fluorescein isothiocyanate; IF-F: Immunofluorescence on fresh frozen tissue.

full house positivity could not be demonstrated. Only IgM showed 2+ mesangial staining and the rest of the immunoglobulins and complements were focal. The EM of this case however revealed numerous predominantly mesangial electron dense deposits along with few subepithelial and subendothelial deposits.

Of 11 cases of membranous nephropathy (MN, 11 cases) diagnostic immunofluorescence with IgG was noted in 8 cases. One case showed weak (1+) staining with characteristic fine granularity and two cases were negative for IgG.

To make the diagnosis of IgA nephropathy (39 cases,

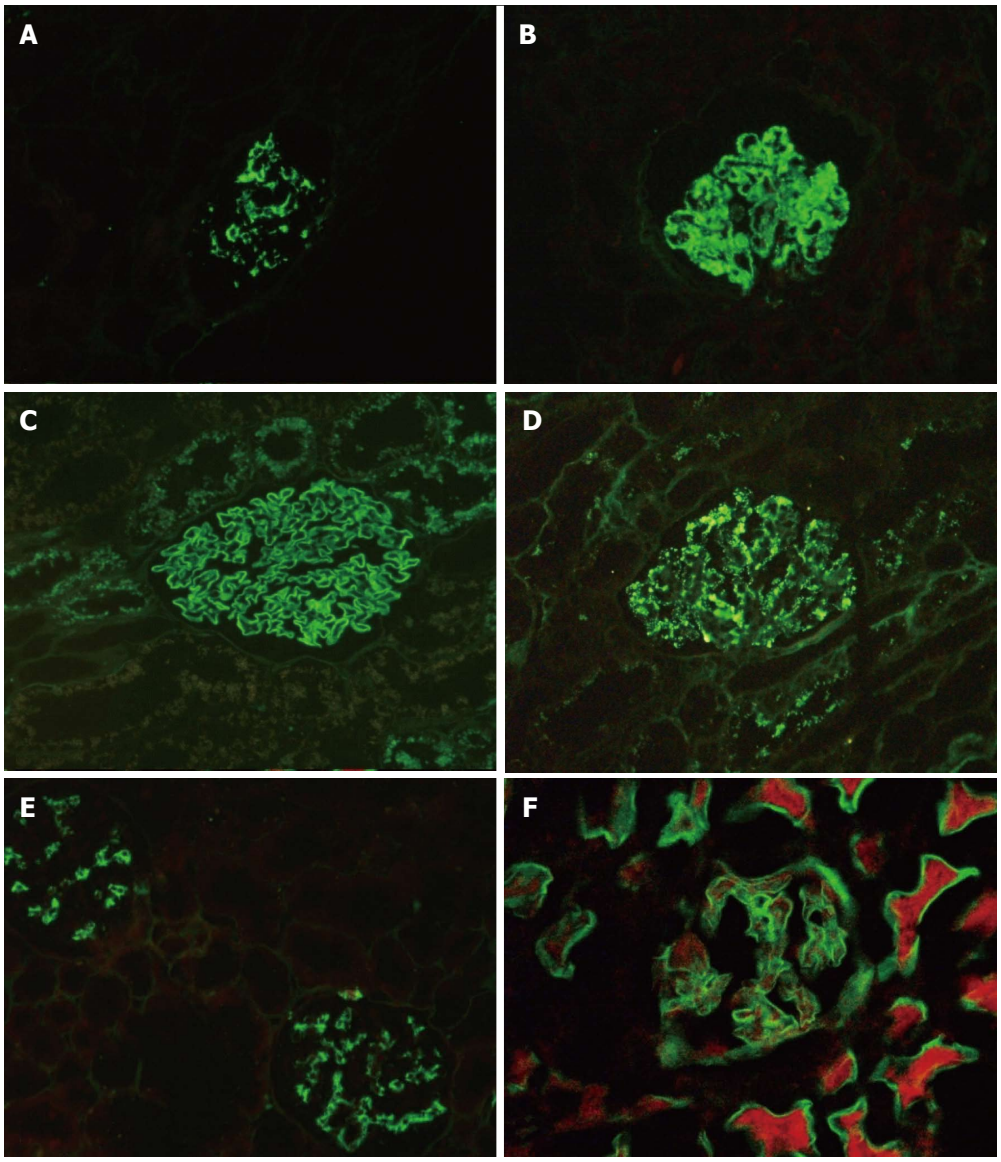

Figure 3 Glomerulonephritis diagnosed on immunofluorescence on paraffin. A: IgA nephropathy: There is predominantly mesangial deposition of IgA (FITC IgA × 100); B: Class IV or diffuse lupus nephritis: Immunofluorescence reveals coarsely granular deposition of immunoglobulins in both mesangium and in the peripheral capillary wall (FITC IgG × 200); C: Membranous nephropathy: Immunofluorescence reveals fine granular capillary wall deposition of IgG (C, FITC IgG × 200); D: Post infectious glomerulonephritis: Garland pattern with elongated peripheral loop deposits is depicted, along with occasional mesangial deposits (D, FITC C3c, × 200); E: C1q nephropathy with mesangial deposition of C1q (FITC C1q × 100); F: Diabetic nephropathy with linear accentuation of glomerular capillary wall and tubular basement membrane (FITC IgG × 200). FITC: Fluorescein isothiocyanate.

16%) or to exclude it in cases with minimal change morphology, mesangial proliferation or focal segmental glomerulosclerosis (64 cases, 26.3%) constituted the bulk of indication for IF-P in our routine practice. In two cases with isolated hematuria, suspected IgA nephropathy and nonspecific light microscopy, IF-P was negative for immunoglobulins which prompted ultrastructural examination of the cases. Classical glomerular basement membrane changes of collagenopathy consistent with Alport syndrome and thin basement membrane disease were identified. IF-P was also performed in patients of diabetic nephropathy with hematuria to exclude secondary IgAN.

In this series there were 8 cases which were diagnosed as chronic glomerulonephritis (CGN), the underlying etiology could be established in 5 cases (IgAN = 3, IC-MPGN = 1 and C-MPGN = 1). The immune complexes could not be demonstrated in 3 cases of chronic glomerulonephritis, one of which was a case of biopsy proven MPGN and the other was a case of IgAN. In one case no immune complexes were seen, however no previous renal biopsy record was available.

In one case of post transplant recurrence of nodular glomerulosclerosis of undetermined cause, IF-P resulted in confirming the diagnosis of light chain deposition disease (LCDD) with kappa restriction[3]. Deposits were identified in the glomerular nodules, tubular basement membranes, arterioles and arteries (Figure 4A and B). Primary amyloidosis was identified in 2 cases demonstrating light chain restriction (Figure 4C and D). Light chains were

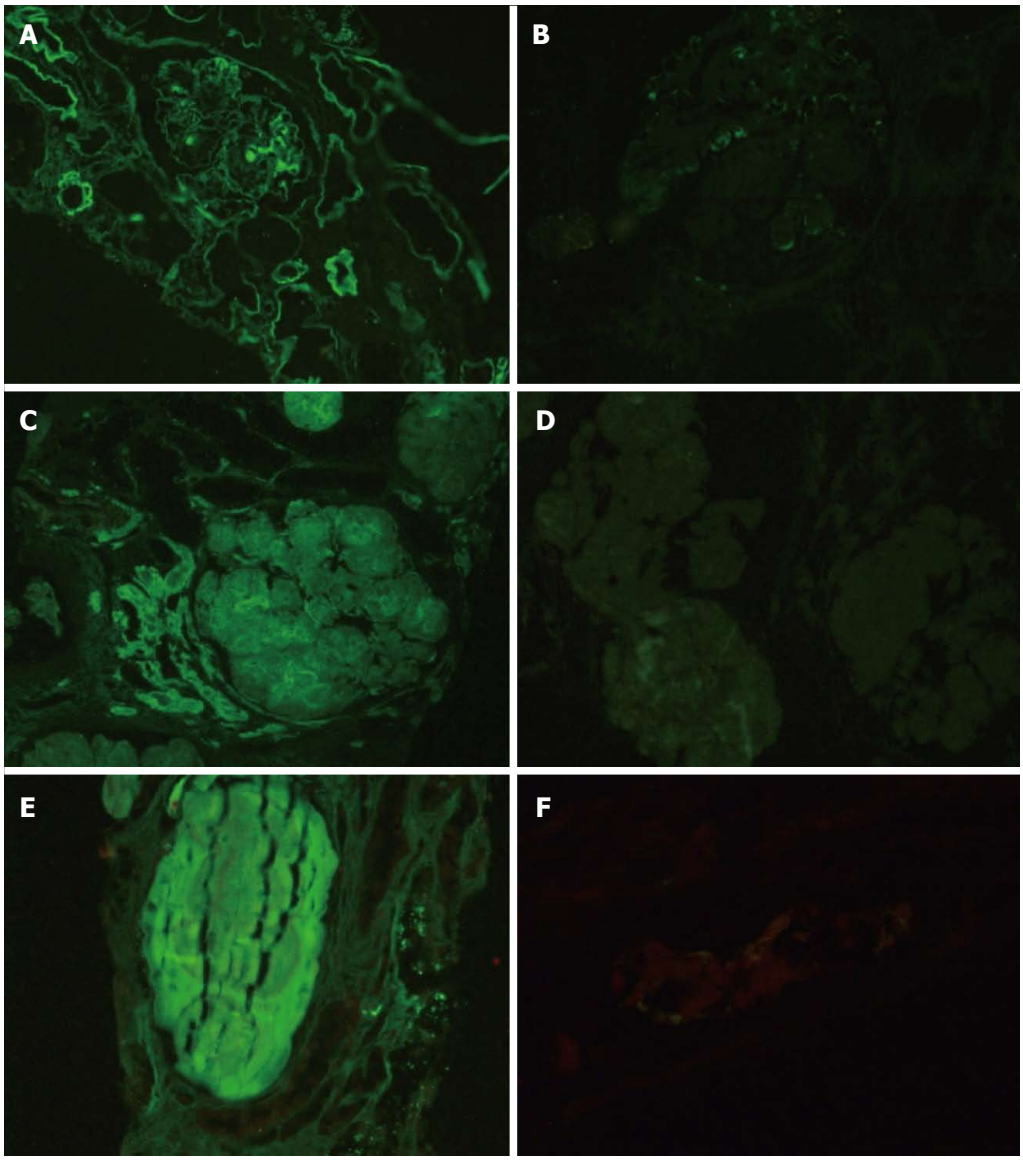

Figure 4 Immunofluorescence on paraffin to demonstrate monoclonal deposits. A case of light chain deposition disease with kappa light chain restriction. There is nodular mesangial, capillary wall and tubular basement membrane deposition of kappa light chain (A, FITC kappa, × 100) while no deposition of lambda is noted (B, FITC lambda, × 200); C: A case of primary amyloidosis with lambda light chain restriction. The lambda deposition is noted in the mesangium (FITC lambda × 200); D: There is no deposition of kappa (D, FITC kappa × 200); E: A case of cast nephropathy with kappa light chain restriction. Note the brightly positive casts for kappa (FITC kappa × 200) with no traces of lambda (F, FITC lambda × 200). FITC: Fluorescein isothiocyanate.

also identified in tubular casts, confirming the diagnosis of cast nephropathy in two cases. One of these cases demonstrated light chain restriction (Figure 4E and F). Other than suspected cast nephropathy, IF-P was performed in cases of primary tubulointersitial disease with significant proteinuria or hematuria to exclude concomitant glomerular disease.

Comparison between immunofluorescence on frozen and paraffin embedded tissue

Comparative IF-F and IF-P was available in 37 cases. Thirty-five of these cases (93.8%) had either equal intensity or a minor difference in intensity of staining (1+) for the diagnostic immunoglobulin/complement. Significant difference was observed in just 2 cases; a case of C-MPGN and a case of MN (Table 3).

Technically assessment of immunofluorescence on the paraffin embedded tissue was found to be less challenging than IF-F with clearly observed morphology and ease of comparison with light microscopic findings.

DISCUSSION

IF studies are integral to diagnostic renal pathology and renal pathologists are often left frustrated by a lack of representative tissue in material sent for routine IF. The technique of IF-F is well established however requires a separate representative core of kidney tissue, a cryostat and technical expertise for satisfactory results. Descriptions of enzyme treatment of formalin fixed

Table 3 Comparison of immunofluorescence intensity on fresh frozen and paraffin embedded renal biopsies

Disease	Number of cases with no difference in intensity of diagnostic immunoglobulin/complement (%) IF-F = IF-P	Number of cases with difference in intensity of diagnostic immunoglobulin/complement (%) IF-F > IF-P		Total number of cases
		Difference of 1 +	Difference of 2 +	
IgA nephropathy	7 (78%)	2 (22%)	-	9
C-MPGN	1 (25%)	2 (50%)	1 (25%)	4
IC-MPGN	4 (100%)	-	-	4
Lupus nephritis	3 (50%)	3 (50%)	-	6
C1q nephropathy	2 (100%)	-	-	2
Membranous nephropathy	3 (43%)	3 (43%)	1 (14%)	7
Post infectious glomerulonephritis	1 (100%)	-	-	1

IF-F: Immunofluorescence on fresh frozen tissue; IF-P: Immunofluorescence on paraffin embedded tissue; C-MPGN: Complement mediated membranoproliferative glomerulonephritis; IC-MPGN: Immune complex mediated membranoproliferative glomerulonephritis.

paraffin embedded (FFPE) tissue followed by IF studies (IF-P) can be found in literature from as early as 1976, however the technique has still not found a place in most renal pathology laboratories[2].

The use of a cross linking fixative like formaldehyde leads to masking of antigens. In addition calcium and other divalent ions form complexes with proteins during fixation and these complexes can block the antigenic determinants[1]. It is to unmask these determinants that enzyme treatment of FFPE tissue is necessary before applying antibodies. In our laboratory the technique of IF-P was standardized using proteinase K and it was applied prospectively as a "salvage" technique with good results.

Proteinase K is an enzyme that exhibits broad substrate specificity. It is isolated from a fungus, Engyodontium album (formerly Tritirachium album) and is able to digest keratin hence the name proteinase "K"[4]. Different proteolytic enzymes including pronase, trypsin and pepsin have been tried in various studies as demonstrated in Table 4[5-15].

As evident from Table 4 multiple studies comparing IF-P and IF-F have clearly established that IF-P is a feasible and valuable "salvage" technique. Comparable staining intensities have been demonstrated for immunoglobulins, with albeit lower sensitivity for detection of complement.

In an early study by Fogazzi *et al*[8] paraffin embedded sections were treated with pronase (0.75 g/L for 60 min) and the fluorescence intensity and location was compared with frozen sections in cases of IgAN (n = 10), membranous nephropathy (n = 8) and proliferative lupus nephritis (n = 10). The diagnostic immunoglobulins were detected with equal or increased intensity in 100% cases with a slightly reduced immunoreactivity for C3 in enzyme treated tissue. Structural details were better assessed in terms of location and morphology of deposits. On retrospective digestion of 1 and 2 year old blocks identical staining patterns were obtained in approximately 86% of cases.

Using a similar protocol as Fogazzi *et al*[8], Nasr *et al*[10] compared IF-F and IF-P in 71 renal biopsies including a spectrum of renal diseases. In glomerular diseases diagnostic findings were obtained in 100% of cases of lupus nephritis, acute post-infectious glomerulonephritis, cryoglobulinemic glomerulonephritis, fibrillary glomerulonephritis, primary amyloidosis, 88% of cases of IgAN, 80% cases of LCDD, 60% of cases of MPGN type 1, 50% cases of idiopathic MN and 20% of cases of anti-glomerular basement membrane (anti-GBM) disease. In all disease categories studied IF-P was less sensitive than IF-F for the detection of C3 similar to Fogazzi *et al*[8]. In addition they found reduced sensitivity for the detection of IgG in cases of MN (50%) and anti-GBM (20%) disease. They also demonstrated utility of the technique in tubulointerstitial diseases such as myeloma cast nephropathy and light chain proximal tubulopathy and found IF-P satisfactory in demonstrating light chain restriction.

More recently Messias *et al*[15] studied paraffin immunofluorescence in 304 native renal biopsies. The false positive staining on the surface and within capillary lumina attributed to sera adsorption secondary to fixation by the authors was also recognized in our cases and was more pronounced in under-digested tissue. They described a novel utility of the technique in evaluating masked paraprotein and immune complex deposits. The light chain crystals in light chain proximal tubulopathy were only demonstrated after enzyme digestion. Out of 61 cases where IF-P was performed to unmask immunoglobulins, it was helpful in 20 cases which included 9 cases of membranous like glomerulopathy with masked IgG-kappa deposits (MGMIDK) a novel entity first described by Larsen *et al*[16], 4 cases of MPGN with light chain restriction and 7 cases of MPGN with mixed essential cryoglobulinemia, which would have been misdiagnosed as C3 glomerulopathy. They recommended that all cases of C3 glomerulopathy based on routine immunofluorescence should be subjected to paraffin immunofluorescence to reach the correct diagnosis and avoid unnecessary investigations into complement abnormalities. In addition any case where the routine immunofluorescence findings do not match the ultrastructural findings should undergo paraffin immunofluorescence. However the authors reiterated, and we concur that IF on paraffin embedded

Table 4 Studies using the technique of immunofluorescence on enzyme digested paraffin embedded tissue in literature

Ref.	Year	Enzyme used	Cases (*n*)	IF panel applied	Significant results
[2]	1976	Trypsin for 120 min	NA	Immunoglobulins and complement	Feasible to demonstrate immunoglobulins but not complement Reduced background immunofluorescence
[5]	1979	Trypsin	52 renal biopsies	IgG, IgA, IgM, C3, Fibrinogen	Accurate detection of immunoglobulins (90%) and complement (75%) in comparison with IF on frozen
[6]	1980	Trypsin	21 (LN, MN, IgAN)	IgG, IgM, IgA	IF on trypsin-digested tissue was as sensitive as IF-F for immunoglobulins but less sensitive for complement
[7]	1980	Pepsin (0.4%) and trypsin	Experimental mice model of anti GBM disease	IgG	Pepsin +/- trypsin digestion better than trypsin alone Enzyme digested tissue showed trivial decrease in sensitivity but good preservation in comparison with IF on frozen
[8]	1989	Pronase (0.75 g/L for 60 min at 37 ℃)	IgAN (10), MN (8), Proliferative LN (10)	IgG, IgA, IgM, C3, C1q	Correct diagnosis possible in all cases Better structural details and less fading of IF Lower intensity staining for C3 Retrospectively performed digestion on 1 and 2 yr old blocks, satisfactory in 86% cases
[9]	2005	Microwave treatment (10 min) followed by Protease Ⅶ (0.05% for 30/60 min) Trypsin (0.25% for 120 min)	IgAN (7), LN (7), MN (7), MPGN (3)	IgG, IgA, IgM, C3	Microwave treatment followed by protease digestion better than trypsin digestion Diagnostic immunoglobulin found in more than 80% cases
[10]	2006	Pronase (0.75 g/L for 60 min at 37 ℃)	MN (8), MPGN (5), LN (5), PIGN (5), IgAN (8), Cryo GN (5), Fibrillary GN (5), Anti GBM (5), Cast nephropathy (5), Amyloid (5), LCDD (5), LCFS (10)	IgG, IgA, IgM, C3, C1q, kappa and lambda	Diagnostic utility in 83% cases Useful in dysproteinemia related renal disease particularly LCFS Less sensitive for staining with C3 in MPGN type I, Cryo GN, PIGN Less sensitive for IgG in MGN and anti-GBM disease
[11]	2007	Proteinase XXIV	LN (5), antiGBM (5), MN (9)	NA	IF-P on proteinase XXIV is more sensitive than IF-P with pronase In LN, better intensity staining for C1q and IgG In anti GBM, 80% sensitivity for detection of IgG In MGN, 55% sensitivity for detection of IgG
[12]	2009	Microwave treatment and/or Proteinase K – (30 or 60 min)	IgAN (24), MN (22), LN (24)	IgG, IgA, IgM, C3	Rate of agreement between immunofluorescence on paraffin sections and immunofluorescence on frozen sections with respect to the presence of IgA was 56.5%, IgM - 44.4%, IgG - 73.9%, and C3 - 51.5% IF-P may be used as a salvage technique when frozen tissue is not available
[13]	2011*	Trypsin (30 min), Pepsin	IgAN (20), MN (25)	IgA, IgG, HBsAg, HbcAg	Trypsin digestion better than pepsin digestion IF-P slightly weaker signal than IF-F
[14]	2012	Heat - Tris/Citrate buffer Pronase RTU (60 min at 37 ℃)	LN (15), MN (11), IgMN (10), MPGN (2), IgAN (2)	IgG, IgA, IgM, C3, C1q	Heat based retrieval using Tris buffer showed superior results Pronase digestion shows less sensitivity for detection of immunoglobulins and complement
[15]	2015	Proteinase K for 20 min	304 cases (207 cases as salvage and 97 cases for antigen unmasking)	IgG, IgA, IgM, C3, C4, C1q, fibrinogen, kappa and lambda	Not only a good salvage technique but prevents misdiagnosis due to masked immune complex or light chain deposition

LN: Lupus nephritis; MN: Membranous nephropathy; IgAN: IgA nephropathy; IgMN: IgM nephropathy; MPGN: Membranoproliferative glomerulonephritis; anti GBM: Anti glomerular basement membrane nephritis; PIGN: Post infectious glomerulonephritis; Cryo GN: Cryoglobulinemic glomerulonephritis; LCDD: Light chain deposition disease; LCFS: Light chain fanconi syndrome; RTU: Ready to use; HBsAg: Hepatitis B surface antigen; HbcAg: Hepatitis B core antigen; IF: Immunofluorescence.

tissue cannot supplant routine IF-F in renal biopsy interpretation.

In the present series we found comparable results for staining with IF-F and IF-P. As described in other studies in a few cases expected immunofluorescence results were not obtained by IF-P, including two cases of membranous nephropathy, two cases of dense deposit disease, one case of lupus nephritis, one case of suspected PIGN and three cases of chronic glomerulonephritis; even in the presence of optimal enzyme digestion. Most of these cases (except PIGN and CGN) had electron microscopic confirmation of presence of electron dense deposits, thus they were truly false negative results. We opine that this variability may be a result of differences in time of exposure of the renal biopsy to formalin, making the unmasking of antigenic

determinants more difficult. This of course becomes a limitation of IF-P in a "salvage" scenario, as a negative result in the presence of optimal digestion would always be questionable; however a positive result will always aid in the diagnosis[3,17].

Nonetheless in the majority of cases undergoing routine fixation and processing, IF-P was successful in providing immunofluorescence results which added to the final diagnosis. Based on our results, we also now offer this technique for skin biopsies and for amyloid characterization in extra renal sites.

Based on the experience in our laboratory, we conclude that immunofluorescence on formalin fixed paraffin embedded tissue is a useful "salvage" technique in case of non-availability of representative fresh frozen tissue; however it is not without pitfalls. Technically assessment of enzyme digestion on each slide is mandatory for accurate interpretation of staining. Antibody staining of under digested tissue can result in both false positive as well as false negative results. Even with optimal digestion expected immunofluorescence results are sometimes not obtained and there is a yet unexplained reduced sensitivity for complement as demonstrated in multiple studies; all of which may result in a misdiagnosis. The extra slices of the renal biopsy taken for IF-P from the paraffin block apart from the routine stains result in insufficient tissue remaining in the block for any further staining or review. Within these limitations, we have demonstrated a significant diagnostic utility of this technique particularly in glomerular diseases and continue to offer it as a "salvage" option.

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We would like to acknowledge Madhusudhan Bhatt, PhD student in our laboratory for his technical assistance and our laboratory attendant Mr. Harish for his sincere work.

COMMENTS

Background

Immunofluorescence (IF) is an indispensible technique for rendering an accurate diagnosis in renal pathology. Diseases such as IgA nephropathy, C1q nephropathy and C3 glomerulopathy cannot be diagnosed without IF. Direct IF on fresh frozen tissue (IF-F) is the most widely used IF technique.

Research frontiers

IF on paraffin embedded renal biopsies after enzyme treatment has not found widespread use in renal pathology laboratories. This leads to incomplete diagnosis and suboptimal patient management.

Innovations and breakthroughs

To overcome the hurdles above, a method of enzymatic digestion of formalin fixed paraffin embedded tissue was standardized and introduced in the authors' laboratory in 2011.

Applications

The authors discussed their experience with this technique in day-to-day diagnostic renal pathology, its utility in reaching final diagnoses and comparing it with usual IF-F where available. Technical and interpretation issues faced are described in detail, and may be helpful to any laboratory planning to introduce this technique.

Peer-review

It is an interesting paper that could be very useful for pathologists or nephrologists involved in renal pathology.

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ORIGINAL ARTICLE

Prospective Study

What is the optimal level of vitamin D in non-dialysis chronic kidney disease population?

Pablo Molina, José L Górriz, Mariola D Molina, Sandra Beltrán, Belén Vizcaíno, Verónica Escudero, Julia Kanter, Ana I Ávila, Jordi Bover, Elvira Fernández, Javier Nieto, Secundino Cigarrán, Enrique Gruss, Gema Fernández-Juárez, Alberto Martínez-Castelao, Juan F Navarro-González, Ramón Romero, Luis M Pallardó

Pablo Molina, Sandra Beltrán, Belén Vizcaíno, Verónica Escudero, Julia Kanter, Ana I Ávila, José L Górriz, Luis M Pallardó, Department of Nephrology, Dr Peset University Hospital, 46017 Valencia, Spain

José L Górriz, Luis M Pallardó, Department of Medicine, Universitat de València, 46005 Valencia, Spain

Mariola D Molina, Department of Mathematics, Universidad de Alicante, San Vicente del Raspeig, 03690 Alicante, Spain

Jordi Bover, Department of Nephrology, Fundació Puigvert, 080348 Barcelona, Spain

Elvira Fernández, Department of Nephrology, Hospital Universitari de Lleida, IRBlleida (Institut de Reserca Biomèdica de Lleida), 25198 Lleida, Spain

Javier Nieto, Department of Nephrology, Hospital General Universitario de Ciudad Real, 13005 Ciudad Real, Spain

Secundino Cigarrán, Department of Nephrology, Hospital da Costa Burela, 7880 Lugo, Spain

Enrique Gruss, Gema Fernández-Juárez, Department of Nephrology, Hospital Universitario Fundación Alcorcón, 28922 Madrid, Spain

Alberto Martínez-Castelao, Department of Nephrology, Hospital Universitario de Beellvitge, IDIBELL, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

Juan F Navarro-González, Research Unit and Department of Nephrology, University Hospital Nuestra Señora de Candelaria, 38010 Santa Cruz de Tenerife, Spain

Ramón Romero, Department of Medicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

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Correspondence to: Pablo Molina, MD, PhD, Department of Nephrology, Dr Peset University Hospital, Avda. Gaspar Aguilar, 90, 46017 Valencia, Spain. molina_pab@gva.es

Telephone: +34-9-61622462
Fax: +34-9-61622462

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Abstract

AIM

To evaluate thresholds for serum 25(OH)D concentrations in relation to death, kidney progression and hospitalization in non-dialysis chronic kidney disease (CKD) population.

METHODS

Four hundred and seventy non-dialysis 3-5 stage CKD patients participating in OSERCE-2 study, a prospective, multicenter, cohort study, were prospectively evaluated and categorized into 3 groups according to 25(OH)D levels at enrollment (less than 20 ng/mL, between 20 and 29 ng/mL, and at or above 30 ng/mL), considering 25(OH)D between 20 and 29 ng/mL as reference group. Association between 25(OH)D levels and death (primary outcome), and time to first hospitalization and renal progression (secondary outcomes) over a 3-year follow-up, were assessed by Kaplan-Meier survival curves and Cox-proportional hazard models. To identify 25(OH)D levels at highest risk for outcomes, receiver operating characteristic (ROC) curves were performed.

RESULTS

Over 29 ± 12 mo of follow-up, 46 (10%) patients dead, 156 (33%) showed kidney progression, and 126 (27%) were hospitalized. After multivariate adjustment, 25(OH)D < 20 ng/mL was an independent predictor of all-cause mortality (HR = 2.33; 95%CI: 1.10-4.91; P = 0.027) and kidney progression (HR = 2.46; 95%CI: 1.63-3.71; P < 0.001), whereas the group with 25(OH)D at or above 30 ng/mL did not have a different hazard for outcomes from the reference group. Hospitalization outcomes were predicted by 25(OH) levels (HR = 0.98; 95%CI: 0.96-1.00; P = 0.027) in the unadjusted Cox proportional hazards model, but not after multivariate adjusting. ROC curves identified 25(OH)D levels at highest risk for death, kidney progression, and hospitalization, at 17.4 ng/mL [area under the curve (AUC) = 0.60; 95%CI: 0.685-0.69; P = 0.027], 18.6 ng/mL (AUC = 0.65; 95%CI: 0.60-0.71; P < 0.001), and 19.0 ng/mL (AUC = 0.56; 95%CI: 0.50-0.62; P = 0.048), respectively.

CONCLUSION

25(OH)D < 20 ng/mL was an independent predictor of death and progression in patients with stage 3-5 CKD, with no additional benefits when patients reached the levels at or above 30 ng/mL suggested as optimal by CKD guidelines.

Key words: Vitamin D; Chronic kidney disease; Mortality; Renal progression; Hospitalization

Core tip: This study examines the prognosis value of 25(OH)D levels on death, chronic kidney disease (CKD) progression, and hospitalization in a cohort of 3-5 stage CKD subjects not on dialysis. The main findings were the predictor value of vitamin D deficiency (< 20 ng/mL), but not insufficiency (< 30 ng/mL), for the 3-year incidence of death and CKD progression, which remained significant after multivariate adjustments. These results could highlight the need for a revision of the current guidelines, which have defined optimal vitamin D status at ⩾ 30 ng/mL based on levels required to suppress parathyroid hormone, as opposed to our study, which evaluates thresholds for serum 25(OH)D concentrations in relation to "hard" endpoints.

Molina P, Górriz JL, Molina MD, Beltrán S, Vizcaíno B, Escudero V, Kanter J, Ávila AI, Bover J, Fernández E, Nieto J, Cigarrán S, Gruss E, Fernández-Juárez G, Martínez-Castelao A, Navarro-González JF, Romero R, Pallardó LM. What is the optimal level of vitamin D in non-dialysis chronic kidney disease population? *World J Nephrol* 2016; 5(5): 471-481 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i5/471.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i5.471

INTRODUCTION

There is a high prevalence of vitamin D (VD) deficiency in all stages of chronic kidney disease (CKD)[1-5]. Observational studies in this population have shown that VD levels correlated with cardiovascular disease and markers of renal injury, including albuminuria[1,6], renal progression[4,6-8], vascular calcification[9,10], left ventricular hypertrophy[9] and mortality[8,11-13]. Moreover, growing evidence supports a potential role for VD receptor activation in suppressing the renin-angiotensin system, reducing proteinuria and ameliorating kidney dysfunction[14-16], showing 25-hydroxyvitamin D [25(OH)D] as an attractive, cheap and feasible treatment target[17]. As a result of these findings, current guidelines have suggested VD supplementation in CKD patients[18-21], increasing VD supplementation rates among this population[22].

Nevertheless, these recommendations are opinion based and the optimal VD levels as well as the upper safe limit of VD intakes remains controversial[23,24]. Based on the inverse relationship between serum concentrations of 25(OH)D and parathyroid hormone (PTH), most current guidelines have defined VD deficiency and insufficiency, as a serum 25(OH)D level of < 20 ng/mL (50 nmol/L) and 20-29 ng/mL (52-72 nmol/L) respectively[18,19], suggesting a serum concentration of 25(OH)D above 30-40 ng/mL

(75-100 nmol/L) to be desirable, levels at which PTH is suppressed to a minimum in its relation to 25(OH)D[25,26]. By contrast, the Institute of Medicine advocates VD repletion as a level of 20 ng/mL [27]. Determining the 25(OH)D target level for optimal health is especially important in CKD population, where overuse of VD leads to hypercalcemia, hypercalciuria and hyperphosphatemia, which could predispose to vascular calcification, nephrolithiasis and reduced glomerular filtration rate[28-30]. All these data suggest an optimal level of VD exists that is neither too high nor too low[31].

Aware of the lack of evidence behind guidelines recommendations, and our concerns about VD oversupplementation, encouraged us to investigate the optimal VD status in non-dialysis CKD patients. The aim of our study was to evaluate thresholds for serum 25(OH)D concentrations in relation to hard end-points such as death, kidney progression and hospitalization in this population.

MATERIALS AND METHODS

Study design and patient selection

OSERCE-2 was a 3-year follow-up prospective, observational, study which enrolled 742 adults with 3 to 5-stage CKD not on dialysis subjects attending 39 centres in Spain, to evaluate the effects of vascular calcifications and CKD-mineral bone disorders on mortality, hospitalization and kidney progression[32]. Inclusion criteria were age $\geqslant$ 18 years and CKD Stages 3-5. Exclusion criteria were acute kidney injury, transplantation, hospitalization in the month previous to the enrollment, and severe comorbidity. In this post-hoc analysis of the OSERCE-2 study, patients on current treatment with active VD (calcitriol, α-calcidol or paricalcitol) were also excluded, so 25(OH)D levels reflected the effect of the exposure to VD.

The study was reviewed and approved by the Dr Peset Hospital Research Ethics Committee. All study participants provided informed written consent prior to study enrollment.

Study protocol and baseline data

The study protocol of the OSERCE-2 study has been previously reported[32]. All patients were assessed at baseline for blood pressure measurement, lateral lumbar, pelvis and hands X-ray, an ankle brachial pressure index (ABPI) determination and laboratory blood sampling. All blood samples were analyzed in a central laboratory, including 25(OH)D, 1,25(OH)$_2$ vitamin D, creatinine, calcium, phosphorus, intact PTH, albumin, and high-sensitive C-reactive protein. 25(OH)D levels were assessed by radioimmunoassay (Biosource), which were transformed to the usual method of reference (DiaSorin Liaison chemiluminescent radioimmunoassay) for improving the comparability of the results, as previously described[32]. To study the renal progression, blood samples for determination of serum creatinine levels were obtained every 12 mo. Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula[33].

Outcomes

Deaths episodes (primary outcome), time to first hospital admission and the appearance of a combined renal end-point, defined as a drop > 30% in eGFR, or beginning of renal replacement therapy (secondary outcomes), were prospectively gathered over a 3-year period[32].

Statistics analysis

Summary statistics were reported as frequencies or percentages, and as mean ± SD, for categorical and quantitative variables, respectively. Skewed quantitative variables were expressed as geometric mean (95%CI), after log transformation. Presence or absence of prominent calcification for Adragao (AS) and Kauppila scores (KS) was reported as AS $\geqslant$ 3 and KS > 6, respectively.

Patients were classified further into 3 groups by 25(OH)D level: < 20 ng/mL (deficiency), 20-29 ng/mL (insufficiency) and $\geqslant$ 30 ng/mL. Comparison of baseline characteristics in these 3 groups was assessed using one-way analysis of variance (ANOVA) for continuous variables, and χ^2 test for trend, for categorical variables. Analysis of variables independently related to 25(OH)D levels was assessed by lineal regression model. To assess the relationship between the odds of VD deficiency and clinical and laboratory baseline characteristics, a stepwise binary logistic regression was performed between 25(OH)D level < 20 or $\geqslant$ 20 ng/mL as dependent variables. PTH and 1,25(OH)D levels were considered as posterior variables to 25(OH)D levels and then they were not introduced in the models, to avoid an overadjustment bias. Twenty-four hours urine proteinuria was not included either because it was available in only 50% of the patients.

Kaplan-Meier analysis and log-rank tests were used to estimate the effects of VD status on all-cause mortality, appearance of the composite renal end-point, and hospitalizations. We then used univariate and multivariate Cox proportional hazard regression models to determine the association of VD levels with various pre-specified outcomes. Patients with 25(OH)D levels between 20 to 29 ng/mL were considered as reference group. Covariates significantly associated in the univariate analysis were entered (forward selection: Likelihood ratio) into the models. The relatively small number of deaths limited the list of adjustment variables that were included in the regression analyses. To identify VD levels at highest risk for outcomes, we performed a receiver operating characteristic (ROC) curve. The value associated with the highest accuracy was considered as the cut-off point for defining an increased risk of death, appearance of the composite renal endpoint, and hospitalization.

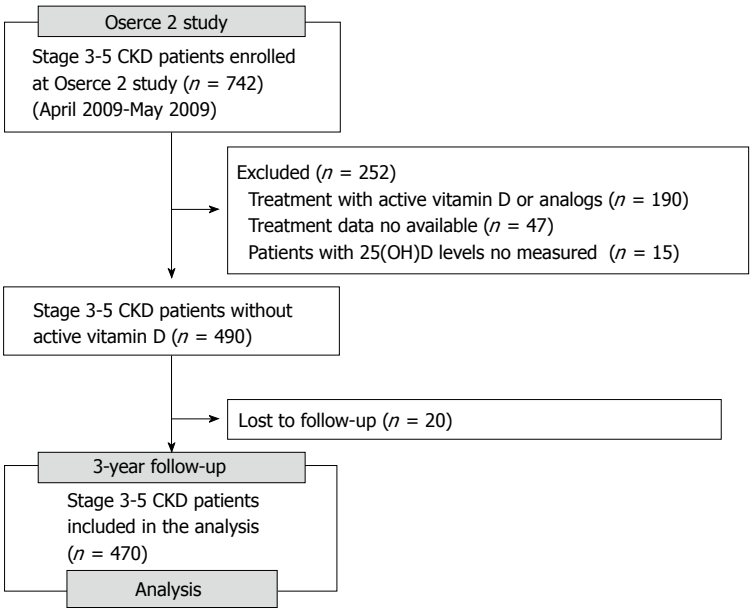

Figure 1 Flow diagram of patient selection for analysis. CKD: Chronic kidney disease; 25(OH)D: 25-hydroxivitamin D.

The literature indicates that annual mortality in patients with stage 3 to 5 CKD (not on dialysis), is between 3% and 9%. Previous studies have shown a 35% prevalence of VD deficiency in this population[3]. Compared with the group with VD deficiency, the group with VD insufficiency shows a 57% decrease in mortality[8]. With 470 patients included, a minimum follow-up of three years, and considering an error of alfa = 0.05, the power estimation of the study is 0.754. The statistical methods of this study were reviewed by MD Molina, from the Department of Mathematics, Universidad de Alicante, Spain, who was included as a co-author. All data analyses were conducted using SPSS, version 15.0 (SPSS Inc., Chicago, IL). A *P*-value < 0.05 was considered statistically significant.

RESULTS

Baseline data

From the 742 subjects enrolled at OSERCE-2 Study, 252 were excluded and 20 were lost to follow-up, leaving 470 patients in the final analysis (Figure 1). Tables 1 and 2 show the patient characteristics and laboratory values, respectively, as a function of vitamin D status. According to 25(OH)D levels, the proportion of patients with deficiency or insufficiency was 53% and 33%, respectively. At baseline, the proportion of patients with 5-stage CKD, diabetes mellitus, diabetic nephropathy and chronic heart failure was higher in the group with less 25(OH)D levels. ABPI, eGFR, PTH, $1,25(OH)_2$ vitamin D and albumin levels were increased in groups with better VD status, which showed lower degree of proteinuria. The group with 25(OH)D less than 20 ng/mL was prescribed more frequently treatment with diuretics and erythropoietin-stimulating agents, with a lower proportion of patients under native VD treatment.

Relationship between 25(OH)D levels and baseline characteristics

Linear correlation analysis showed significant correlation between 25(OH)D levels and eGFR (R = 0.10; *P* = 0.027), body mass index (R = -0.10; *P* = 0.046), serum levels of albumin (R = 0.10; *P* = 0.027), calcium (R = 0.12; *P* = 0.013), $1,25(OH)_2$ vitamin D (R = 0.20; *P* < 0.001), PTH (R = -0.26; *P* < 0.001), and hemoglobin (R = 0.13; *P* = 0.005), proteinuria (log transformed, R = -0.19; *P* = 0.004) and ABPI (R = 0.15; *P* = 0.002). Multivariate binary logistic regression analysis showed as independent predictors of 25(OH) < 20 ng/mL the albumin levels (OR = 0.61; 95%CI: 0.40-0.92; *P* = 0.018), the ABPI (OR = 0.28; 95%CI: 0.11-0.73; *P* = 0.010), and treatment with native VD (OR = 0.35; 95%CI: 0.17-0.73; *P* = 0.005), and diuretics (OR = 2.03; 95%CI: 1.35-3.06; *P* = 0.001).

Mortality

Forty-six (10%) patients died after a mean follow-up of 29 ± 12 mo. Cardiovascular disease (*n* = 16, 35%) and infections (*n* = 8, 17%) were the most common causes of death. Tumors and others accounted for 11% (*n* = 5) and 13% (*n* = 6) of deaths, respectively. In 11 cases (24%) the cause of death was not identified. The Kaplan-Meier survival analysis (Figure 2A) suggested that patients with 25(OH)D less than 20 ng/mL had significantly higher mortality than the other two groups (log rank test, *P* = 0.031). Univariate Cox regression found a more than twice higher risk of death in the group with the 25(OH)D level less than 20 ng/mL compared with the reference group (HR = 2.47; 95%CI: 1.18-5.18; *P* = 0.017), whereas the group with 25(OH)D at or above 30 ng/mL was not significantly different from that with the 25(OH)D between 20 to 29 ng/mL (HR = 0.78; 95%CI: 0.26-2.32; *P* = 0.650). Multivariate analysis

Table 1 Baseline patient characteristics (n = 470), as a function of vitamin D status

	All	25(OH)D < 20 ng/mL	25(OH)D 20-29 ng/mL	25(OH)D ⩾ 30 ng/mL	*P*
n	470	252 (53%)	154 (33%)	64 (14%)	
Age (yr)	66.1 ± 12.9	65.8 ± 13.1	65.9 ± 11.9	68.1 ± 12.1	0.421
Male sex (%)	309 (66%)	162 (64%)	101 (66%)	46 (72%)	0.303
High blood pressure (%)	444 (95%)	242 (96%)	144 (94%)	58 (91%)	0.072
Dyslipidemia (%)	311 (66%)	168 (68%)	101 (66%)	42 (66%)	0.646
Diabetes mellitus (%)	183 (39%)	114 (45%)	53 (34%)	16 (25%)	0.001
Ischemic heart disease (%)	104 (22%)	60 (24%)	33 (22%)	11 (17%)	0.224
Chronic heart failure (%)	43 (9%)	33 (13%)	7 (5%)	3 (5%)	0.005
Stroke (%)	52 (11%)	30 (12%)	15 (10%)	7 (11%)	0.668
Peripheral arterial disease (%)	93 (20%)	59 (24%)	22 (14%)	12 (19%)	0.117
Stage of CKD (%)					
3 (eGFR = 30-59 mL/min per 1.73 m^2)	221 (47%)	103 (41%)	84 (54%)	34 (53%)	0.002
4 (eGFR = 15-29 mL/min per 1.73 m^2)	205 (44%)	105 (46%)	64 (42%)	26 (41%)	
5 (eGFR < 15 mL/min per 1.73 m^2)	44 (9%)	34 (13%)	6 (4%)	4 (6%)	
Etiology of CKD (%)					
Hypertension	108 (23%)	54 (21%)	40 (26%)	14 (22%)	0.039
Diabetes mellitus	108 (23%)	72 (29%)	29 (19%)	7 (11%)	
Tubulointerstitial disease	65 (14%)	24 (10%)	25 (16%)	16 (25%)	
Glomerulonephritis	47 (10%)	26 (10%)	15 (10%)	6 (10%)	
Unknown/others	142 (30%)	75 (30%)	44 (29%)	20 (32%)	
Smoking (%)[1]					
Never	231 (53%)	124 (52%)	82 (58%)	25 (44%)	0.494
Ex-smoker	144 (33%)	81 (34%)	44 (31%)	19 (33%)	
Active	64 (14%)	35 (14%)	16 (11%)	13 (23%)	
Blood pressure (kPa)					
Systolic	19.0 ± 2.9	19.3 ± 2.9	18.6 ± 2.8	19.0 ± 3.1	0.085
Diastolic	10.2 ± 1.5	10.2 ± 1.6	10.1 ± 1.4	10.3 ± 1.7	0.617
Pulse pressure (kPa)	8.8 ± 2.5	9.1 ± 2.5	8.5 ± 2.5	8.7 ± 2.5	0.098
Body mass index (kg/m^2)	28.6 ± 5.1	28.8 ± 5.5	28.6 ± 4.6	27.7 ± 4.4	0.294
Underweight (⩽ 18.5)	6 (1%)	4 (2%)	1 (1%)	1 (2%)	0.353
Normal (18.6-24.9)	96 (20%)	50 (20%)	30 (19%)	16 (25%)	
Overweight (25.0-29.9)	210 (45%)	111 (44%)	68 (44%)	31 (48%)	
Obesity (> 29.9)	158 (34%)	87 (34%)	55 (36%)	16 (25%)	
Waist (cm)					
Males	102.2 ± 12.0	102.1 ± 13.0	102.2 ± 10.6	102.4 ± 11.5	0.989
Females	97.8 ± 13.5	98.3 ± 14.7	97.7 ± 12.2	95.7 ± 11.4	0.760
ABPI	1.01 ± 0.21	0.98 ± 0.20	1.04 ± 0.21	1.05 ± 0.22	0.013
Abnormal ABPI[2]	194 (41%)	100 (41%)	66 (44%)	28 (44%)	0.539
Abnormal Kauppila score[3]	107 (29%)	52 (27%)	35 (29%)	20 (36%)	0.183
Abnormal Adragao score[4]	121 (32%)	66 (33%)	38 (30%)	17 (29%)	0.474
Vitamin D supplementation (%)	43 (9%)	16 (6%)	17 (11%)	10 (16%)	0.012
Use of phosphate binders (%)	72 (15%)	47 (19%)	16 (11%)	9 (14%)	0.105
Use of ACEI/ARB (%)	365 (78%)	196 (79%)	121 (82%)	48 (76%)	0.947
Use of diuretic (%)	287 (61%)	173 (70%)	88 (58%)	26 (42%)	< 0.001
Use of ESA (%)	124 (26%)	77 (31%)	31 (20%)	16 (25%)	0.015

[1]Data available in 439 patientes; [2]< 0.9 or > 1.3; [3]> 6 data available in 370 patients; [4]⩾ 3 data available in 383 patients. If not indicated otherwise, results are presented as mean ± SD, or number (percent). ABPI: Ankle-brachial pressure index; ACEI: Angiotensin-converting-enzyme inhibitor; ARB: Angiotensin II receptor blocker; eGFR: Estimated glomerular filtration rate; ESA: Erythropoietin-Stimulating agents; 25(OH)D: 25-hydroxivitamin D.

showed the predictive value of 25(OH)D levels as a continuous variable for preventing death when adjusted for multiple covariates in different models (Table 3). Adjusted for age, comorbidity, diabetes mellitus, eGFR and phosphorous and albumin levels, the HR for all-cause mortality for 25(OH)D < 20 *vs* 20-29 was 2.33 (95%CI: 1.10-4.91; P = 0.027; Figure 3). The 25(OH)D ⩾ 30 group did not have a significantly different mortality hazard from the reference group (HR = 1.19; 95%CI: 0.37-3.81; P = 0.775).

Progression of CKD and renal replacement therapy initiation

During the follow-up, 81 (17%) patients started renal replacement therapy and 156 (33%) patients showed the composite renal end-point. Kaplan-Meier analysis (Figure 2B) showed that the 25(OH)D < 20 group had significantly more risk than the other two groups (log rank test, P < 0.001). Univariate Cox regression found again higher risk of the renal end-point with 25(OH)D level less than 20 ng/mL compared with 20 to 29 ng/mL (HR = 2.78; 95%CI: 1.84-4.16; P < 0.001), whereas the group with 25(OH)D above 30 ng/mL did not show different risk from reference group (HR = 1.13; 95%CI: 0.59-2.13; P = 0.717). Multivariate analysis showed the predictive value of VD levels as a continuous variable for preventing appearance of renal end point when adjusted for multiple covariates (Table 4). Adjusted for

Table 2 Baseline laboratory values, as a function of vitamin D status

	All (*n* = 470)	25(OH)D < 20 ng/mL (*n* = 252)	25(OH)D 20-29 ng/mL (*n* = 154)	25(OH)D ≥ 30 ng/mL (*n* = 64)	*P*
25-hydroxivitamin D (nmol/L)	52 ± 21	36 ± 9	61 ± 7	90 ± 16	< 0.001
1,25(OH)$_2$ vitamin D (pmol/L)	103 ± 28	97 ± 27	111 ± 28	107 ± 23	< 0.001
Ca_{alb} (mmol/L)	2.40 ± 0.20	2.40 ± 0.15	2.42 ± 0.23	2.45 ± 0.23	0.163
P (mmol/L)	1.10 ± 0.26	1.10 ± 0.26	1.10 ± 0.26	1.07 ± 0.26	0.517
iPTH (ng/L)[1]	91 (85-97)	106 (96-116)	81 (73-91)	64 (55-74)	< 0.001
Creatinine (μmol/L)	221 ± 97	239 ± 106	212 ± 88	212 ± 88	0.017
eGFR (MDRD, mL/min per 1.73 m^2)	29.4 ± 11.5	28.1 ± 11.9	30.8 ± 10.8	30.5 ± 11.1	0.049
Urine protein excretion (g/24 h)[1,2]	0.592 (0.502-0.697)	0.699 (0.573-0.853)	0.448 (0.321-0.626)	0.448 (0.271-0.742)	0.034
hsCRP (nmol/L)[1]	36.2 (29.5-39.1)	37.1 (33.3-41.0)	36.2 (31.4-41.0)	32.4 (26.7-39.1)	0.506
Albumin (g/L)	40 ± 5	39 ± 5	41 ± 5	40 ± 5	0.011
Total proteins (g/L)	77 ± 12	77 ± 11	77 ± 13	76 ± 14	0.877
Total cholesterol (mmol/L)	4.7 ± 1.1	4.7 ± 1.1	4.7 ± 1.0	4.8 ± 1.1	0.603
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.4	0.973
LDL cholesterol (mmol/L)	2.7 ± 0.9	2.7 ± 0.9	2.7 ± 0.9	2.9 ± 0.8	0.344
Hemoglobin (g/L)	130 ± 16	129 ± 16	132 ± 16	132 ± 18	0.058
Ferritin (pmol/L)[1]	225 (207-245)	227 (202-252)	216 (187-252)	247 (191-319)	0.635
Transferrin (μmol/L)	3.0 ± 1.2	2.9 ± 1.2	3.0 ± 1.2	3.1 ± 1.3	0.289
Glucose (mmol/L)	6.3 ± 2.2	6.3 ± 2.3	6.4 ± 2.4	5.9 ± 1.5	0.241

[1]24 h urine proteinuria obtained in 237 (50%) patients; [2]Skewed values are presented as geometric mean with 95%CI. Ca_{alb}: Calcium adjusted for albumin levels; eGFR: Estimated glomerular filtration rate; HSCRP: High-sensitive C reactive protein; iPTH: Intact parathyroid hormone; MDRD: Modification of diet in renal disease; P: Phosphorous; 25(OH)D: 25-hydroxivitamin D. If not indicated otherwise, results are presented as mean ± SD.

Table 3 Adjusted Cox proportional hazards models of patient survival (events = 46)

Model	Covariates controlled for	Adjusted HR (95%CI)	*P*
0 (Unadjusted)	25-hydroxivitamin D levels (mg/dL)	0.95 (0.91-0.99)	0.009
1	25-hydroxivitamin D levels (mg/dL) + age	0.95 (0.91-0.99)	0.009
2	Model 1 + diabetes mellitus, ischemic heart disease, chronic heart failure	0.96 (0.92-0.99)	0.028
3	Model 1 + peripheral arterial disease, abnormal ABPI[1], phosphorous (mg/dL)	0.95 (0.92-0.99)	0.023
4	Model 1 + DBP (mm Hg), 1,25(OH)$_2$ vitamin D (pg/mL), estimated GFR (mL/min per 1.73 m^2)	0.96 (0.92-0.99)	0.020
5	Model 1 + vascular calcification [Kauppila score (log), Adragao score (log)], CKD stage 5	0.95 (0.91-1.00)	0.050
6	Model 1 + obesity, hemoglobin (g/L), albumin (g/dL)	0.95 (0.92-0.99)	0.019

[1]< 0.9 or >1.3. ABPI: Ankle-brachial pressure index; DBP: Diastolic blood pressure; GFR: Glomerular filtration rate.

Table 4 Multivariate Cox regression analysis in relation to renal end point (events = 156)

	HR (95%CI)	*P* value
25-hydroxivitamin D (ng/mL)	0.97 (0.95-0.99)	0.004
Age (yr)	0.99 (0.97-1.00)	0.044
Male sex	2.20 (1.47-3.30)	< 0.001
Estimated GFR (mL/min per 1.73 m^2)	0.93 (0.91-0.95)	< 0.001
ABPI (mmHg)	0.23 (0.10-0.53)	0.001
Hemoglobin (g/L)	0.84 (0.78-0.94)	0.001

ABPI: Ankle-brachial pressure index; GFR: Glomerular filtration rate.

age, gender, diabetes mellitus, eGFR, and phosphorous levels, the HR for the composite renal end-point for the 25(OH)D < 20 group compared to the reference group was 2.46 (95%CI: 1.63-3.71; *P* < 0.001; Figure 4). The 25(OH)D ⩾ 30 group did not have a significantly different hazard for kidney progression from the reference group (HR = 1.20; 95%CI: 0.62-2.32; *P* = 0.581).

Hospitalization

During the follow-up, 126 (27%) patients were admitted for hospitalization, cardiovascular (49%) and infections (20%) being the most common causes. Kaplan-Meier analysis (Figure 2C) indicated that crude hospitalization event-free period was different between the VD groups (log rank test, *P* = 0.039). Univariate Cox regression found a shorter hospitalization event-free period in patients with 25(OH)D level less than 20 ng/mL compared with 20-29 ng/mL (HR = 1.58; 95%CI: 1.05-2.36; *P* = 0.027), with no difference between the 25(OH)D ⩾ 30 and the reference groups (*P* = 0.861). Hospitalization outcomes were predicted by 25(OH) levels (HR = 0.98; 95%CI: 0.96-1.00; *P* = 0.027) in the unadjusted Cox proportional hazards model, but not after adjusting for age, eGFR, diabetes and comorbidity.

Cutoff points to define VD sufficiency based on hard endpoints

ROC curves identified VD levels at highest risk for death,

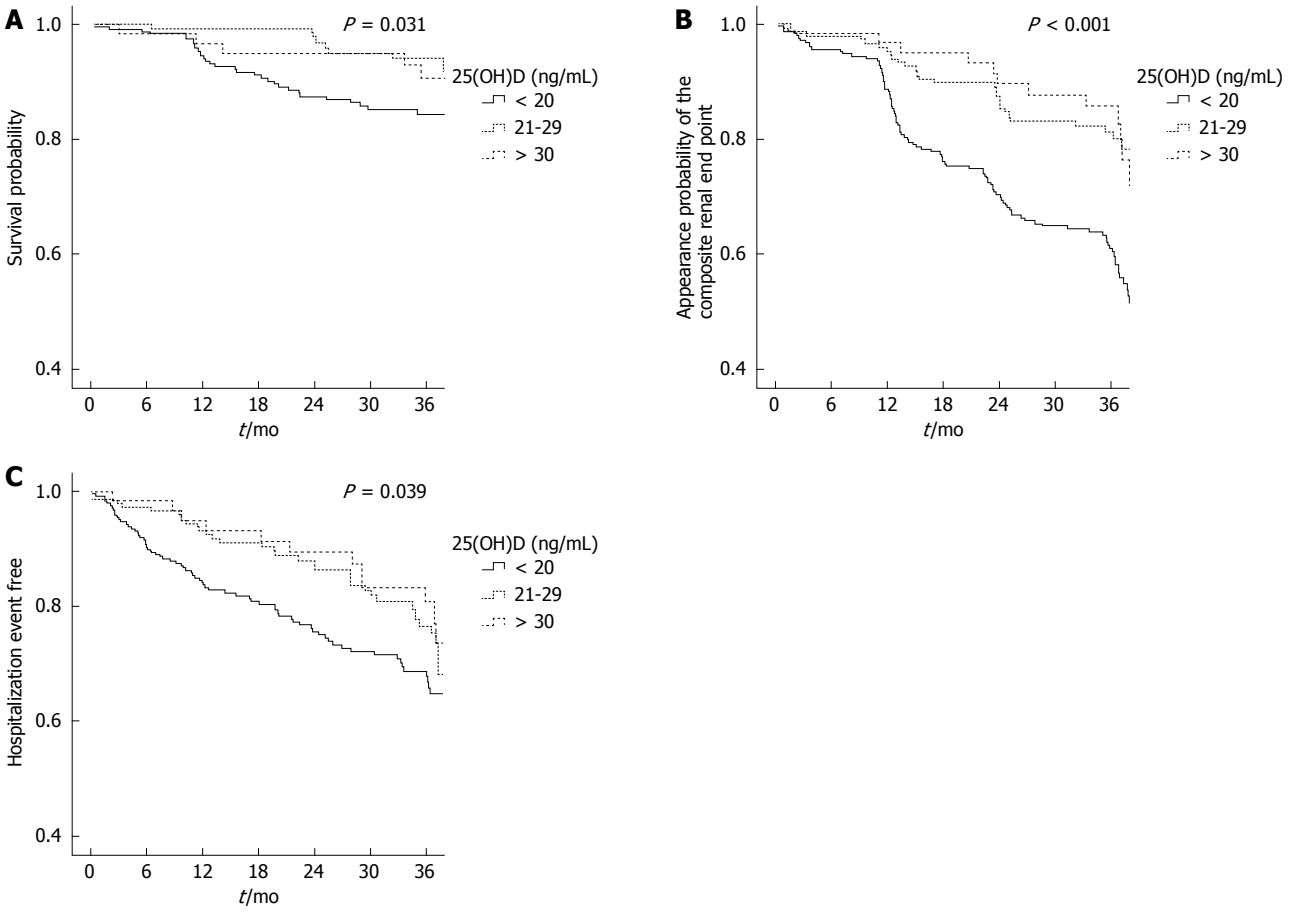

Figure 2 Kaplan-Meier survival (A), and appearance of the composite renal endpoint (B) and the hospitalization (C) curves as a function of 25-hydroxyvitamin D levels (< 20 ng/mL, 20-29 ng/mL and ⩾ 30 ng/mL).

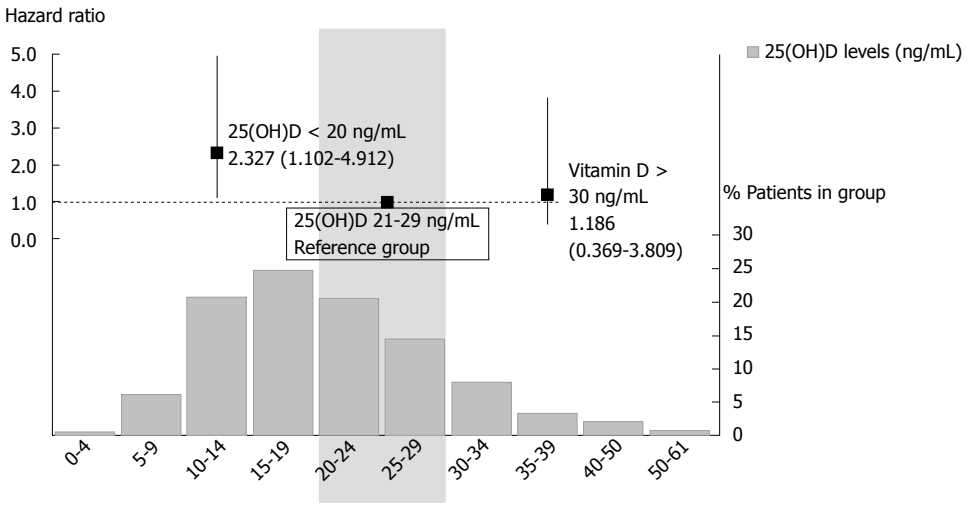

Figure 3 Proportion of patients with different 25-hydroxyvitamin D levels and hazard ratio (95%CI) for mortality after adjustment by age, comorbidity, diabetes mellitus, estimated glomerular filtration rate and albumin levels. 25(OH)D: 25-hydroxivitamin D.

the composite renal endpoint, and hospitalization, at 17.4 ng/mL [area under the curve (AUC) = 0.60; 95%CI: 0.52-0.69; P = 0.027], 18.6 (AUC = 0.65; 95%CI: 0.60-0.71; P < 0.001), and 19.0 (AUC = 0.56; 95%CI: 0.50-0.62; P = 0.048), respectively.

DISCUSSION

One of the main limitations for the development of evidence-based clinical recommendations for VD supplementation lies in the discrepancies in the criteria

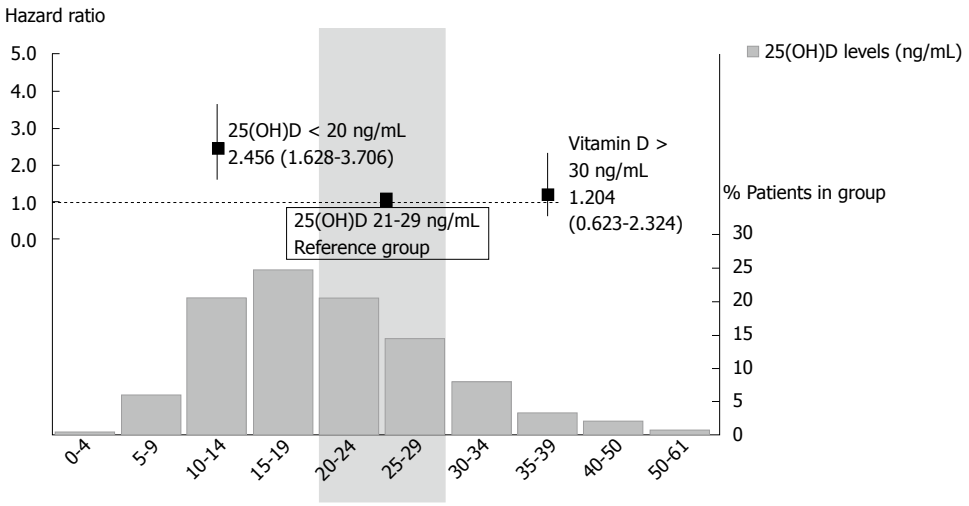

Figure 4 Proportion of patients with different 25-hydroxyvitamin D levels and hazard ratio (95%CI) for composite renal end-point after adjustment by age, sex, diabetes mellitus, estimated glomerular filtration rate and albumin levels. 25(OH)D: 25-hydroxivitamin D.

for defining VD deficiency and insufficiency, which can explain conflicting results from meta-analysis addressing vitamin D levels and outcomes[24,34]. These criteria vary among authors and societies, including 25(OH)D levels below which osteomalacia [10 ng/mL (25 nmol/L)] or secondary hyperparathyroidism [20-30 ng/mL (50 to 75 nmol/L)] may appear[18-20,35,36]. Being aware of their potential clinical significance, the present study examines the prognosis value of 25(OH)D levels in a cohort of 3-5 stage CKD subjects not on dialysis, trying to identify cut-off points for serum 25(OH)D levels to define VD sufficiency. These cut-offs were not based on biological abnormalities as classically noted[25,26], but on VD levels at highest risk for death, CKD progression and all-cause hospitalization.

Although randomized clinical trials are the best way for generating a high evidence for treatment decisions, trials are rare and suboptimal in nephrology[37]. Therefore, observational studies have an important role, particularly when the intervention, in this case vitamin D supplementation, is inexpensive and potentially effective. Although there are previous prospective observational studies which examined the prognosis value of 25(OH)D levels in CKD subjects not on dialysis[8,12], this is the first one, to our knowledge, in which 25OH(D) levels unequivocally reflect exposure to VD, given that patients on treatment with active VD were excluded, as well as including the biggest cohort of non-dialysis CKD subjects with data regarding emerging cardiovascular risk factors as vascular calcification scores and ABPI. In the main analysis of the OSERCE-2 study, low VD levels were associated to worse survival and CKD progression only in the univariate analysis[32]. However, 26% of patients of the study received activated VD, which may confer a protective effect and therefore may decrease any negative effect of VD levels observed, as it has been stated on dialysis population[38]. In this context, we conducted this post-hoc analysis of the OSERCE-2 dataset in patients without active VD treatment. In this selected cohort, the main findings were the independent predictor value of VD deficiency, but not insufficiency, for the 3-year incidence of death and CKD progression, which remained significant after multivariate adjustments, as previously published[8,12]. In a prospective study involving 94 CKD patients, those with 25(OH)D levels less than 16.7 ng/mL had a higher mortality rate[12]. 25(OH)D was confirmed as an independent inverse predictor of death in a 6-year follow-up study which included 168 CKD subjects[8]. In that study patients with ⩾ 15 ng/mL of 25(OH)D showed a reduction in mortality by 33% to 60% in the different models, compared to patients with 25(OH)D < 15 ng/mL. Less CKD progression to end-stage renal disease was also reported in the groups of patients with better VD status. All these data are in agreement with our results, which show how low 25(OH)D levels predicted mortality and CKD progression independently of such traditional and non-traditional risk factors, as vascular calcification or inflammation. In this context, it is noteworthy that the lack of association between 25(OH)D levels and vascular calcification observed in our study, is in agreement with some[12], but not all[9,10], previously published data. These findings indicate that 25(OH)D may impact on CKD outcomes by additional mechanisms including the suppression of the renin-angiotensin system, albuminuria reduction or amelioration of left ventricular hypertrophy[6,9,16,31,39]. Of note, we have detected ABPI as an independent predictor of VD deficiency, which could contribute to vascular stiffness and high cardiovascular risk for this population.

More interestingly, our study, as the first prospective which analyzed the upper level associated to better improvement in survival and CKD progression on CKD patients, did not demonstrate additional benefits on these hard outcomes when patients reached the optimal target levels for VD suggested by current guidelines (⩾ 30 ng/mL). It is noteworthy that all three cut-off points

for serum 25(OH)D levels at highest risk for death, CKD progression and all-cause hospitalization were between 17 ng/mL and 19, which reinforces the threshold value for abnormally reduced 25(OH)D in 20 ng/mL. These findings confirm the data reported in the biggest retrospective observational study analyzing VD and mortality in CKD patients. Navaneethan *et al*[40] studied 12763 patients with 3-4 stage CKD, showing 25(OH)D level ⩽ 15 ng/mL to be associated independently with a 33% increased risk of all-cause mortality, whereas the group with 25(OH)D levels of 15-29 ng/mL did not show a significantly increased risk of mortality compared with patients with 25(OH)D levels ⩾ 30 ng/mL.

Taking all these data together, we agree with the Institute of Medicine recommendation to consider sufficient 25(OH)D levels of at least 20 ng/mL, given that serum 25(OH)D concentrations above 30 ng/mL are not consistently associated with increased benefit[27,40]. In addition, most clinical trials have only confirmed the neutral effect of VD supplementation on hard outcomes[41], whereas some controlled studies have shown positive results in spite of the mean VD concentration not reaching the optimal recommended levels of ⩾ 30 ng/mL[16]. Moreover, VD might not be safe in all settings, and supplementing could cause harm in people with CKD, who have a high prevalence of vascular calcification, and a decreasing ability for renal excretion of calcium and phosphorous[32,42]. Excessive VD supplementation may be particularly harmful in those high risk individuals with serum 25(OH)D levels above 20 ng/mL which are classified as insufficient according to current guidelines, and who then are treated with high-dose supplements of VD containing many times the levels of intake recommended for adults (600-800 UI/d)[18,27,43]. Although some experts suggest that it is safe to carry higher vitamin D levels (40-70 ng/mL), this recommendation is based on acute and not long-term observations[44].

Lastly, our study confirmed the high prevalence of low VD status on CKD patients[1-5]. There are many factors which could contribute to the deficiency that are not related to GFR, including limited exposure to the sun, reduced dietary intake and urinary loss of 25(OH)D and VD-binding protein in proteinuric nephropathies[24,44,45]. The present study, as others[8,12,38], has shown significant correlation between 25(OH)D levels and body mass index and albumin, which emphasizes the relationship between nutritional status, VD levels and survival in chronic illness as CKD. Of note, the independent relationship observed, even after adjustment for chronic heart failure, between VD deficiency and diuretic use. VD deficiency is highly prevalent in heart failure patients, being a significant predictor of reduced survival. In addition, loop diuretics treatment may worsen osteoporosis on general population, but no data are available in CKD patients[46,47].

Strengths and limitations

The strong points of the study include the relatively high number of patients included and the 3-year follow-up, which strengthens the study's power. To minimize the inter-method and seasonal variability in VD and PTH measurements, blood samples were analyzed by a central laboratory, and patients' recruitment was done in a short period of time (April-May)[32]. In contrast, there are several limitations to be commented. As a longitudinal study, it is still insufficient to determine whether the association between low 25(OH)D levels and worse CKD outcomes is causal and reversible, which should be tested in future randomized clinical trials. The results may not be valid to non-Caucasian populations living at other latitudes, or to patients on active VD treatment. The multivariate analysis of cardiovascular deaths was limited due to its low incidence. Lastly, it would be interesting to study other relevant bone-related clinical outcomes, such as bone-density changes or fracture risk.

In conclusion, in accordance with previously published data, the present study confirms: (1) a high prevalence of 25(OH)D deficiency and insufficiency in non-dialysis CKD patients; and (2) an independent association between serum 25(OH)D levels and worse clinical outcomes, such as death and CKD progression. The results of this study add to the knowledge of optimal VD status in non-dialysis CKD patients, identifying the threshold value for abnormally reduced 25(OH)D in 20 ng/mL, which is in agreement with the Institute of Medicine recommendations. Whereas high doses of VD supplementation on this population can lead to a calcium and phosphate overload, promoting vascular calcification and CKD progression, our results suggest that, with the limitations inherent to the observational studies, 25(OH)D levels between 20 to 30 ng/mL could be sufficient for CKD patients. Randomized clinical trials are warranted to know the most favorable 25(OH)D level for CKD patients.

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COMMENTS

Background

Although knowledge of the skeletal and non-skeletal effects of nutritional vitamin D (VD) has expanded, no consensus currently exists within the medical community regarding the criteria for defining thresholds for VD supplementation in chronic kidney disease (CKD) patients.

Research frontiers

Based on levels of 25(OH)D required to suppress parathyroid hormone

(PTH), clinical guidelines most commonly recommend a serum concentration of 25(OH)D above 30-40 ng/mL (75-100 nmol/L), levels at which PTH is suppressed to a minimum in its relation to 25(OH)D. However, there is a lack of evidence regarding this target recommendation, and overuse of VD supplementation on this population can lead to a calcium and phosphate overload, promoting vascular calcification and CKD progression.

Innovations and breakthroughs

Being aware of both the therapeutic and iatrogenic power of VD supplementation, the present study examines the prognosis value of 25(OH)D levels in a cohort of 3-5 stage CKD subjects not on dialysis, trying to identify cut-off points for serum 25(OH)D levels to define VD sufficiency. These cut-offs were not based on biochemical abnormalities as classically noted, but on VD levels at highest risk for death, CKD progression and all-cause hospitalization. The results of this study add to the knowledge of optimal VD status in non-dialysis CKD patients, identifying the threshold value for abnormally reduced 25(OH)D in 20 ng/mL.

Applications

The data in this study suggested that the optimal VD level might be lower than is currently recommended, advocating that 25(OH)D levels at or above 20 ng/mL could be sufficient for CKD patients. The authors recommend caution when nutritional VD is prescribed.

Terminology

25(OH)D, also known as calcifediol, is a prehormone that is produced in the liver by hydroxylation of vitamin D3 (cholecalciferol). Serum 25(OH)D levels are considered the best indicator of VD status.

Peer-review

The paper with the title: "What is the optimal level of vitamin D in non-dialysis CKD population?" is an interesting well written article and the authors claim that their study as the first prospective which analyzed the upper level of VD associated to better improvement in survival and CKD progression on CKD patients, did not demonstrate additional benefits on these hard outcomes when patients reached the optimal target levels for VD suggested by current guidelines (≥ 30 ng/mL).So with this study, despite the limitations, the authors provide a new option in this so controversial field of VD treatment in CKD patients.

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META-ANALYSIS

Association of blood transfusion with acute kidney injury after transcatheter aortic valve replacement: A meta-analysis

Charat Thongprayoon, Wisit Cheungpasitporn, Erin A Gillaspie, Kevin L Greason, Kianoush B Kashani

Charat Thongprayoon, Wisit Cheungpasitporn, Kianoush B Kashani, Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MN 55905, United States

Erin A Gillaspie, Kevin L Greason, Division of Cardiovascular Surgery, Department of Surgery, Mayo Clinic, Rochester, MN 55905, United States

Kianoush B Kashani, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Mayo Clinic, Rochester, MN 55905, United States

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Correspondence to: Kianoush B Kashani, MD, Assistant Professor, Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States. kashani.kianoush@mayo.edu
Telephone: +1-507-2667093
Fax: +1-507-2667891

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Abstract

AIM

To assess red blood cell (RBC) transfusion effects on acute kidney injury (AKI) after transcatheter aortic valve replacement (TAVR).

METHODS

A literature search was performed using MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and clinicaltrials.gov from the inception of the databases through December 2015. Studies that reported relative risk, odds ratio or hazard ratio comparing the risks of AKI following TAVR in patients who received periprocedural RBC transfusion were included. Pooled risk ratio (RR) and 95%CI were calculated using a random-effect, generic inverse variance method.

RESULTS

Sixteen cohort studies with 4690 patients were included in the analyses to assess the risk of AKI after TAVR in patients who received a periprocedural RBC transfusion. The pooled RR of AKI after TAVR in patients who received a periprocedural RBC transfusion was 1.95 (95%CI: 1.56-2.43) when compared with the patients who did not receive a RBC transfusion. The meta-analysis was

then limited to only studies with adjusted analysis for confounders assessing the risk of AKI after TAVR; the pooled RR of AKI in patients who received periprocedural RBC transfusion was 1.85 (95%CI: 1.29-2.67).

CONCLUSION

Our meta-analysis demonstrates an association between periprocedural RBC transfusion and a higher risk of AKI after TAVR. Future studies are required to assess the risks of severe AKI after TAVR requiring renal replacement therapy and mortality in the patients who received periprocedural RBC transfusion.

Key words: Acute kidney injury; Transcatheter aortic valve replacement; Meta-analysis; Mortality; Blood transfusion

Core tip: We performed this meta-analysis to assess the impact of periprocedural red blood cell (RBC) transfusion on the risk of acute kidney injury (AKI) after transcatheter aortic valve replacement (TAVR). We verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion. This study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

Thongprayoon C, Cheungpasitporn W, Gillaspie EA, Greason KL, Kashani KB. Association of blood transfusion with acute kidney injury after transcatheter aortic valve replacement: A meta-analysis. *World J Nephrol* 2016; 5(5): 482-488 Available from: URL: http://www.wjgnet.com/2220-6124/full/v5/i5/482.htm DOI: http://dx.doi.org/10.5527/wjn.v5.i5.482

INTRODUCTION

Patients with severe symptomatic aortic stenosis have destitute prognosis with medical treatment alone[1]. Transcatheter aortic valve replacement (TAVR), also known as transcatheter aortic valve implantation, is an exciting new approach to the treatment of high-risk or inoperable patients with severe aortic stenosis[2-6]. Despite advances in TAVR procedures, acute kidney injury (AKI) is one of the most frequent complications of TAVR, ranging in the literature from 15% to 57%[3,7-9]. Notably, the subset of patients, who develop AKI after TAVR, also have a high mortality rate of 9%-44% at 30 d and 32%-56% at 1 year[7,8].

Perioperative anemia has been shown to be independently associated with AKI after cardiac surgery[10,11]. Anemia can result in decreased renal oxygen delivery, increased oxidative stress and impaired hemostasis[10]. Thus, perioperative red blood cell (RBC) transfusion is used to improve oxygen delivery. However, stored RBC transfusion can also promote a pro-inflammatory state, impair tissue oxygen delivery, and induce tissue oxidative stress[12,13]. The association of AKI with RBC transfusion after TAVR is conflicting. While a few studies have demonstrated a higher incidence of AKI among patients who received periprocedural RBC transfusion[14-23], the others have shown no such association[24-29]. Thus, we conducted this meta-analysis to assess the impact of periprocedural RBC transfusion on the risk of AKI after TAVR.

MATERIALS AND METHODS

Search strategy

Two investigators (Thongprayoon C and Cheungpasitporn W) independently searched published studies and conference abstracts indexed in MEDLINE, EMBASE, Cochrane Database of Systematic Reviews and clinicaltrials.gov from the inception of the databases through December 2015. The search strategy used is described in the supplementary material. A manual search for additional relevant studies using the references from these retrieved articles was also performed. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for a systematic review and meta-analysis[30].

Inclusion criteria

We included studies that: (1) enrolled adult ($\geqslant$ 18 years old) patients; (2) provided information about periprocedural RBC transfusion and comparator patients who did not receive RBC transfusion; (3) included AKI after TAVR as an outcome; (4) were randomized clinical trials or observational studies (case-control, cross-sectional or cohort studies) published as original studies or conference abstracts; and (5) provided data to calculate odds ratios (ORs), relative risks, hazard ratios (HRs) or standardized incidence ratios with 95%CIs. No language limits were applied.

Study eligibility was independently determined by the two investigators noted previously. Differing decisions were resolved by mutual consensus. The quality of each study was evaluated using the Newcastle-Ottawa quality assessment scale[31].

Data extraction

A standardized data collection form was used to extract the following information: Last name of the first author, article title, study design, year of study, country of origin, year of publication, sample size, AKI definition, blood transfusion, confounder adjustment, and the adjusted effect estimate with 95%CI.

Statistical analysis

Review Manager software (Version 5.3, Copenhagen, Denmark) from the Cochrane Collaboration was used for data analysis. Point estimates and standard errors were extracted from individual studies and were combined by the generic inverse variance method of DerSimonian

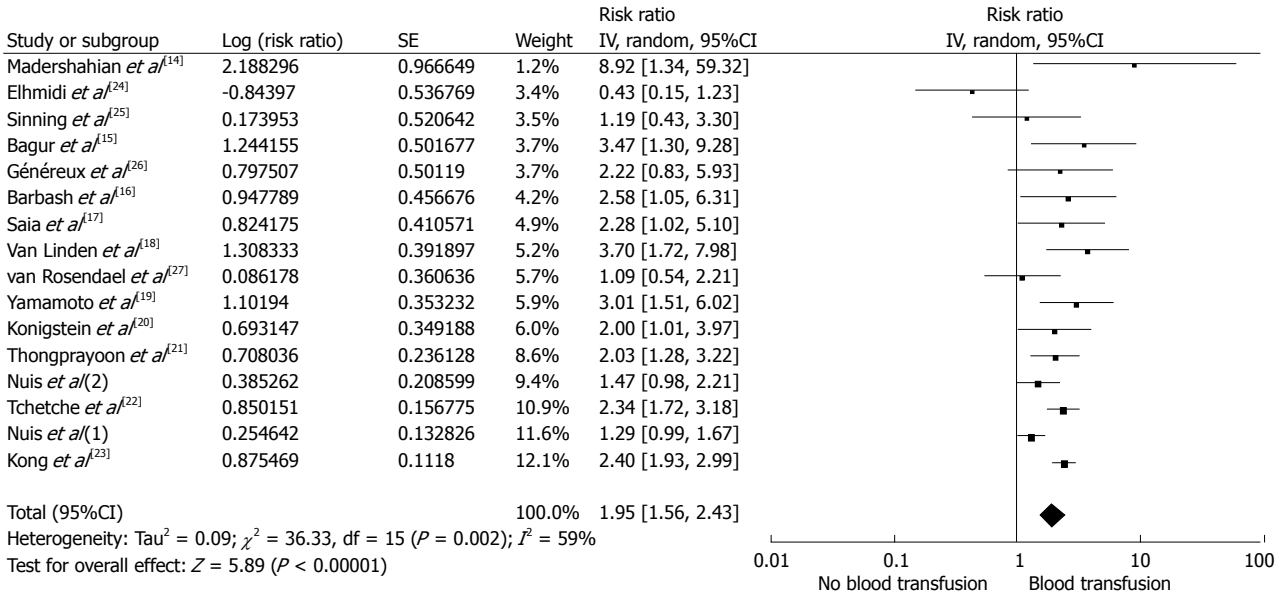

Figure 1 Forest plot of comparing the risk of acute kidney injury after transcatheter aortic valve replacement in patients who received red blood cell transfusion and those who did not. Square data markers represent risk ratios (RRs); horizontal lines, the 95%CIs with marker size reflecting the statistical weight of the study using random-effects meta-analysis. A diamond data marker represents the overall RR and 95%CI for the outcome of interest.

and Laird[32]. Given the high likelihood of between-study variances, a random-effect model was used. Statistical heterogeneity was assessed using Cochran's Q test. This statistic was complemented with the I^2 statistic, which quantifies the proportion of the total variation across studies that is due to heterogeneity rather than chance. An I^2 of 0%-25% represents insignificant heterogeneity, 26%-50% low heterogeneity, 51%-75% moderate heterogeneity and > 75% high heterogeneity[33]. The presence of publication bias was assessed by funnel plots of the logarithm of ORs *vs* their standard errors[34].

RESULTS

Our search strategy yielded 1327 articles. Of these, 1169 articles were excluded based on their relevance and the eligibility criteria, following the review of their title and abstract. The remaining 158 articles underwent full-length review and an additional 142 were excluded for failing to meet the criteria: 114 articles did not report the outcome of interest; and 28 articles were not observational studies or radomized clinical trials. Sixteen cohort studies were included in the meta-analysis to assess the risk of AKI after TAVR in patients who received periprocedural RBC transfusion (Table 1). Of the 16 cohort studies, eight studies performed adjusted analysis for known risk factors for AKI[14,15,19,20,23,24,28,29]. Supplementary Item 2 outlines our search methodology and selection process.

Study quality

All observational studies were considered moderate to high quality, with a median Newcastle-Ottawa quality assessment scale of 6.5 (range: 6-8) as shown in Table 1.

AKI definition

All included studies identified the AKI occurrence, based on the change in serum creatinine (SCr) or GFR after TAVR. One of the included studies also used urine output criteria for the AKI diagnosis[25]. Twelve[16,17,19-23,25-29] of the 16 included studies used standard AKI definitions (modified Risk, Injury, Failure, Loss of kidney function[35]; Acute Kidney Injury Network[36]; or Kidney Disease Improving Global Outcomes criteria[37]), as shown in Table 1.

AKI risk

The pooled risk ratio (RR) of AKI following TAVR in patients who received a RBC transfusion was 1.95 (95%CI: 1.56-2.43; I^2 = 59%). Figure 1 shows the forest plot of the included studies. When meta-analysis was limited to the studies using standard AKI definitions, the pooled RRs were 1.89 (95%CI: 1.55-2.31; I^2 = 50%). To minimize the effects of confounders, we performed a sensitivity analysis and excluded studies that did not include an adjusted analysis for known risk factors for AKI. The pooled RR of AKI after TAVR remained significant in patients who received periprocedural RBC transfusions (RR = 1.85; 95%CI: 1.29-2.67; I^2 = 75%), shown in Figure 2.

Nuis *et al*[28] assessed the dose response relationship of a RBC transfusion and AKI, and demonstrated an increased risk of AKI with a higher number of RBC transfusions with ORs of 1.47 (95%CI: 0.98-2.22), 3.05 (95%CI: 1.24-7.53), 4.81 (95%CI: 1.45-15.95) for 1-2 units, 3-4 units, and ⩾ 5 units of RBC transfusion, respectively. Reporting of severe AKI requiring renal replacement therapy (RRT) was limited. Van Linden *et al*[18] reported a higher risk of AKI requiring RRT with an OR of 8.8 (95%CI: 1.7-45.6; Table 1).

Table 1 Main characteristics of the studies included in this meta-analysis

Ref.	Country[1]	Year	*n*	Transfusion definition	AKI definition (changes in baseline)	RR for AKI	Confounder adjustment	S, C, O[2]
Sinning *et al*[25]	Germany	2010	77	RBC in 2 d post-procedure	Increase in SCr of ⩾ 0.3 mg/dL or ≥ 50% or U output < 0.5 mL/kg per hour for > 6 h in 48 h post procedure	1.19 (0.43-3.31)	None	3, 0, 3
Bagur *et al*[15]	Canada	2010	213	Peri-procedural blood	Decrease in eGFR of > 25% at 48 h post procedure or hemodialysis needed during index hospitalization	3.47 (1.30-9.29)	HTN, COPD	3, 1, 3
Van Linden *et al*[18]	Germany	2011	261	Blood > 4 u in 7 d post-operative	Decrease in eGFR of > 25% or increase in SCr of 50% in 7 d post procedure	AKI 3.7 (1.7-7.9) RRT 8.8 (1.7-45.6)	None	3, 0, 3
Nuis *et al*[29]	Netherlands	2011	118	Peri-procedural RBC	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	1.29 (1.01-1.70)	Previous MI, leukocyte count, logistic EuroScore	3, 1, 3
Elhmidi et al[24]	Germany	2011	234	Post-operative blood	Decrease in eGFR of > 25% or increase in SCr of 50% in 7 d post procedure	0.43 (0.15-1.23)	Baseline creatinine, STS score, DM	3, 2, 3
Madershahian *et al*[14]	Germany	2012	50	RBC	Increase in SCr of ⩾ 0.5 mg/dL or ⩾ 25% from baseline within 48 h post procedure	8.92 (1.34-59.26)	COPD and contrast amount	3, 1, 3
Kong *et al*[23]	Australia	2012	52	Peri-procedural RBC	SCr criteria of RIFLE classification in 48 h post procedure	2.4 (2.0-3.1)	TA, history of HTN	3, 1, 3
Tchetche *et al*[22]	France, Netherlands, Italy	2012	743	RBC	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	2.34 (1.72-3.18)	None	3, 0, 3
Barbash *et al*[16]	United States	2012	165	Post procedure blood	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	2.58 (1.05-6.29)	None	3, 0, 3
Nuis *et al*[28]	Netherlands, Canada, Germany, Belgium, Columbia	2012	995	RBC in 24 h post procedure	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	1-2 u, 1.47 (0.98-2.22); 3-4 u, 3.05 (1.24-7.53); ⩾ 5 u, 4.81 (1.45-15.95)	PVD, CHF, maximal leukocyte count, logistic EuroScore	3, 2, 3
Saia *et al*[17]	Italy	2013	102	Peri-procedural RBC	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	2.28 (1.02-5.10)	None	3, 0, 3
Konigstein *et al*[20]	Israel	2013	251	Blood	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	2.00 (1.01-3.97)	Gender, HTN, DM, dyslipidemia, PVD, CHF, stroke, COPD, PHTN, VC, CKD, valve type and size	3, 2, 3
Yamamoto *et al*[19]	France	2013	415	RBC	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 72 h post procedure	3.01 (1.54-6.15)	Contrast amount and LVEF	3, 1, 3
Généreux *et al*[26]	United States	2013	218	Blood	VARC-modified RIFLE stage 2 or 3 until discharge	2.22 (0.83-5.92)	None	3, 0, 3
Thongprayoon *et al*[21]	United States	2015	386	Intra-operative RBC	Increase in SCr of ⩾ 0.3 mg/dL in 48 h or ⩾ 50% in 7 d post procedure	2.03 (1.28-3.23)	None	3, 0, 3
van Rosendael *et al*[27]	Netherlands	2015	210	Peri-procedural RBC	Increase in SCr of ⩾ 0.3 mg/dL or ⩾ 50% in 7 d post procedure	1.09 (0.54-2.22)	None	3, 0, 3

[1]Countrys are listed by their three letter country code; [2]Quality Assessment Newcastle-Ottawa scale: S: Selection; C: Comparability; O: Outcome. AKI: Acute kidney injury; CHF: Congestive heart failure; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; HTN: Hypertension; LVEF: Left ventricular ejection fraction; MI: Myocardial infraction; PHTN: Pulmonary hypertension; PVD: Peripheral vascular disease; RIFLE: Risk, injury, failure, loss of kidney function, and end-stage kidney disease; SCr: Serum creatinine; STS: Society of thoracic surgeons; TA: Transapical approach; VARC: Valve academic research consortium; VC: Vascular complication; RBC: Red blood cell.

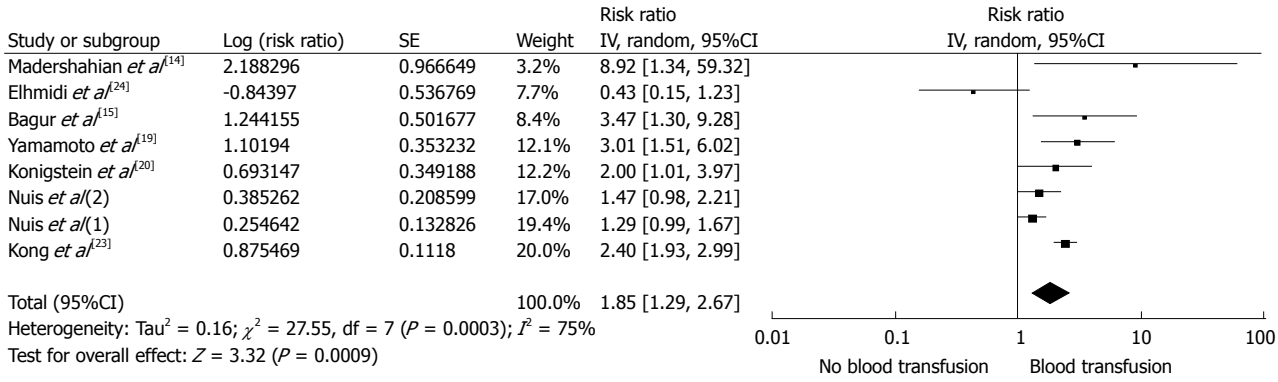

Figure 2 Forest plot of the adjusted analysis comparing the risk of acute kidney injury after transcatheter aortic valve replacement in patients who received red blood cell transfusion and those who did not. The square data markers represent represent risk ratios (RRs); horizontal lines, the 95%CIs with marker size reflecting the statistical weight of the study using random-effects meta-analysis. A diamond data marker represents the overall RR and 95%CI for the outcome of interest.

Evaluation for publication bias

Funnel plots to evaluate publication bias for the risk of AKI after TAVR in patients who received a RBC transfusion are summarized in Supplementary Figures 1 and 2. The graphs demonstrate no obvious asymmetry and, thus, suggest an insignificant publication bias.

DISCUSSION

In this meta-analysis, we verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion. This association remained significant when adjusting for potential confounders.

The mechanism for the higher incidence of AKI after TAVR in patients with a periprocedural RBC transfusion is not well-elucidated. Analysis has shown that preserved RBCs used in transfusions undergo progressive structural and functional changes during storage, such as reduced deformability and increased tendency to aggregate. These changes result in the deterioration of RBC function and viability, and the resultant accumulation of free iron and pro-inflammatory agents[38] leads to AKI[8,39-41]. Studies have also shown an association between RBC transfusions and increased leukocyte count in patients who developed AKI after TAVR[28,29].

Nuis *et al*[28] reported that the number of RBC transfusions was an independent predictor of AKI following TAVR. In their study, a higher number of RBC transfusions were found to be associated with a higher AKI incidence. Interestingly, the investigators did not find significant associations between AKI and the clinical indications for transfusion (*i.e.*, baseline anemia, bleeding complications, or blood loss).

The risks of transfusion-associated AKI is not limited to TAVR; patients undergoing coronary artery bypass grafting or surgical aortic valve replacements who require transfusions also have a higher frequency of AKI[41-43].

Although the included studies in our meta-analysis were all of moderate to high quality, there are some limitations of this study that bear mentioning. First, there were statistical heterogeneities among the enrolled studies. The potential sources of these heterogeneities include the variations in the diagnostic methodology of AKI after TAVR and the differences in confounder adjustment methods. Second, the data on severe AKI requiring RRT after TAVR is lacking. Further studies are certainly warranted to further delineate the impact of transfusions after TAVR with specific regard to the severity of AKI. Third, the data on valve size and approaches for TAVR procedure were limited. These factors might have affected the risk of AKI following TAVR. Lastly, this is a meta-analysis of observational studies with the inherent limitation that a causal relationship cannot be inferred.

The threshold for transfusions is constantly changing. The deleterious effects of transfusions are well documented, and many institutions have worked hard to create protocols to diminish unnecessary transfusions. Our meta-analysis demonstrates an association between periprocedural RBC transfusion and a higher risk of AKI following TAVR. In many cases, patients undergoing TAVR have considerable debility and comorbid conditions. This study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

COMMENTS

Background

Transcatheter aortic valve replacement (TAVR) is an exciting new approach to the treatment of high-risk or inoperable patients with severe aortic stenosis. Despite advances in TAVR procedures, acute kidney injury (AKI) is one of the most frequent complications of TAVR, associated with significant morbidity and mortality following the procedures.

Research frontiers

The association of AKI with red blood cell (RBC) transfusion after TAVR is conflicting in the findings of previous literature. It is thus necessary to assess the impact of periprocedural RBC transfusion on the risk of AKI after TAVR.

Innovations and breakthroughs

In this study, the authors verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion.

Applications

The data in this study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

Terminology

PRISMA: Preferred reporting items for systematic reviews and meta-analyses, *etc.*

Peer-review

This is a reasonable first meta-analysis of association of blood transfusion with AKI after transcatheter aortic valve replacement. The results have potential clinical applications.

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