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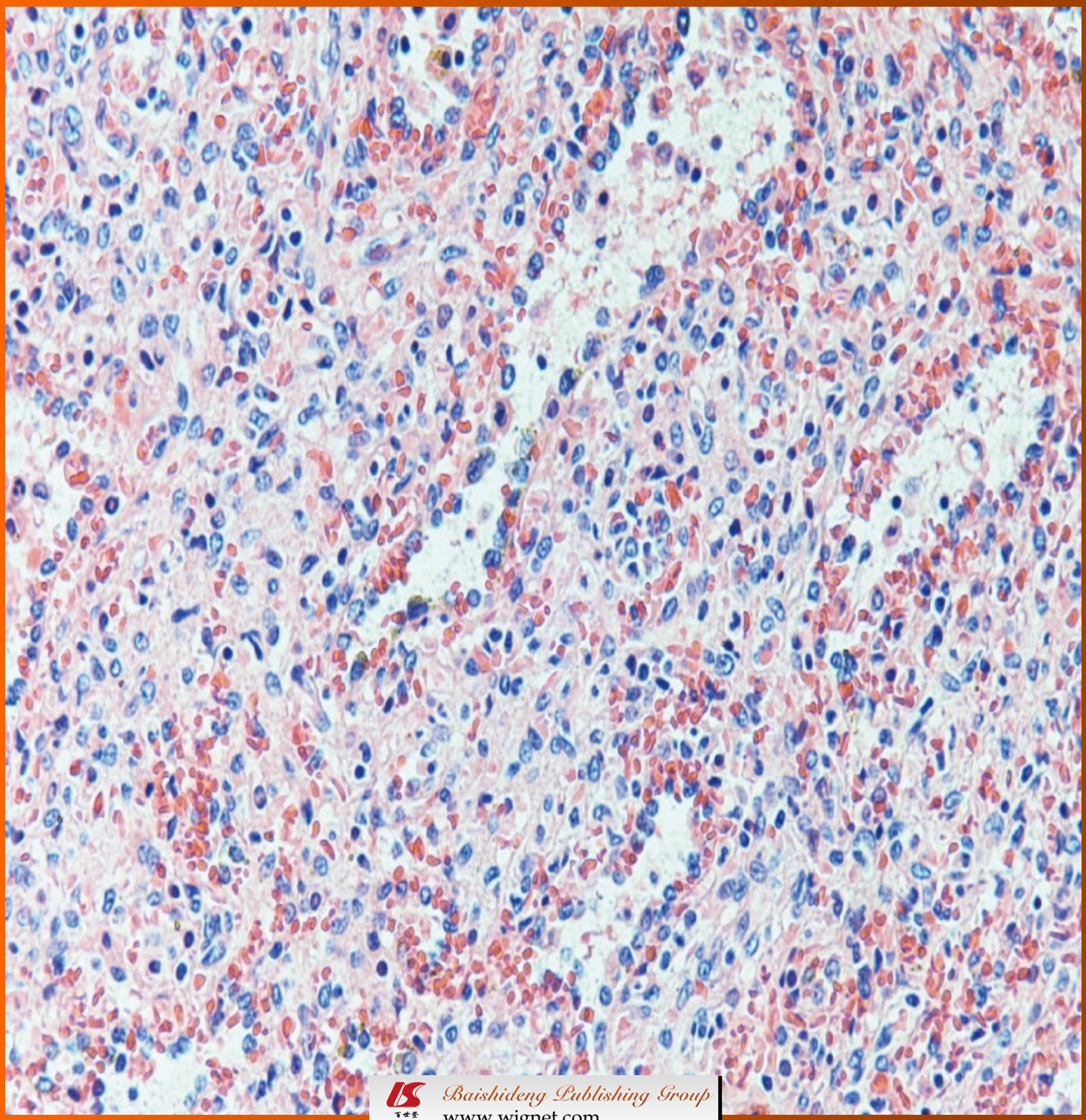

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EDITORIAL

Mechanisms of *Helicobacter pylori* antibiotic resistance: An updated appraisal

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Abstract

Helicobacter pylori (*H. pylori*) antibiotic resistance is the main factor affecting the efficacy of the current eradicating therapies. The aim of this editorial is to report on the recent information about the mechanisms accounting for the resistance to the different antibiotics currently utilized in *H. pylori* eradicating treatments. Different mechanisms of resistance to clarithromycin, metronidazole, quinolones, amoxicillin and tetracycline are accurately detailed (point mutations, redox intracellular potential, pump efflux systems, membrane permeability) on the basis of the most recent data available from the literature. The next hope for the future is that by improving the knowledge of resistance mechanisms, the elaboration of rational and efficacious associations for the treatment of the infection will be possible. Another auspicious progress might be the possibility of a cheap, feasible and reliable laboratory test to predict the outcome of a therapeutic scheme.

INTRODUCTION

The discovery of *Helicobacter pylori* (*H. pylori*) infection and its role in different diseases from chronic gastritis to gastric cancer has radically changed the management of patients with this condition. Unfortunately, the goal of achieving a cure in all treated patients at the first therapeutic approach, as generally occurs for common infective diseases, has not been achieved for *H. pylori*. Indeed, it has been immediately evident that only a few antibiotics are active against such a bacterium in the acidic environment of the stomach.

The initial susceptibility of *H. pylori* to both clarithromycin and imidazoles, key drugs for triple first-line therapies, has progressively been undergoing a marked reduction and the eradication rate following therapy regimens including these antibiotics is decreasing[1]. Similarly, the low *H. pylori* resistance rate towards quinolones, mainly used for second-line therapy, observed in the past has increased during the last decade, whilst both amoxicillin and tetracycline resistance rates seem to have remained low[2].

Alternative molecules, such as furazolidone, bismuth salts and rifabutin are not available worldwide and they are not free of significant side-effects. All these observations highlight the crucial role of antibiotic resistance in the management of *H. pylori* infection[3,4]. Therefore, the knowledge of resistance mechanisms may contribute to elaborate more rational antibiotic combinations with the aim of improving treatment success.

We reviewed the mechanisms of *H. pylori* antibiotic resistance towards the drugs mainly used, including clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline.

CLARITHROMYCIN

Clarithromycin remains the currently available most powerful antibiotic against *H. pylori* with minimal inhibitor concentrations (MICs) being the lowest as compared to the other molecules. Indeed, MIC values as low as 0.016-0.5 mg/L are generally reported, antibiotic resistance being recognized with MIC values ⩾ 1.0 mg/l (range: 2-256 mg/L)[5,6]. The bacteriostatic activity of clarithromycin depends on its capacity to inhibit the protein synthesis by binding to the 50S bacterial ribosomal subunit. Extensive studies by PCR-based tools have demonstrated that point mutations in the peptidyltransferase region encoded in domain V of 23S rRNA are responsible for the bacterial resistance to macrolides[7]. These mutations are able to inhibit the binding between clarithromycin and the ribosomal subunit dedicated to the specific antibiotic related protein synthesis[7,8]. The more frequent mutations associated with clarithromycin resistance are the transition adenine to cytosine in 2143 and 2142 positions of rRNA, whilst the substitution of adenine with cytosine in 2142 position is less frequent. These mutational events are responsible for more than 90% of clarithromycin resistance in developed countries[9]. In detail, the mutation at position 2143 seems to be associated with different resistance levels rather than an on/off behavior, with MIC values widely ranging from 0.016 to 256 mg/L. Conversely, the mutations at position 2142 are associated with more restricted MIC values, close to 64 mg/L[10,11]. Of note, we found that the presence of the A2143G point mutation, rather than the A2142G or A2142C mutation, markedly reduces *H. pylori* eradication rate[12]. These data should suggest that a mutational event detected *in vitro* does not precisely predict *in vivo* results[13].

Several other point mutations have been identified such as A2115G, G2141A, T2117C and T2182C, T2289C, G224A, C2245T, C2611A. Besides the low frequency, the clinical relevance of the A2115G, G2141A, T2117C T2289C, G224A, C2245T mutation is still not proven, their role not being consistently reported[14,15], whilst the T2182C and C2611A have been associated with low resistance levels[16,17].

Another relevant mechanism for macrolide resistance is ascribed to the efflux pump system. At least 5 conserved families of drug efflux mechanisms are associated to bacterial species, including Small Multidrug Resistance, Multidrug and Toxic Compounds Extrusion proteins, the Major Facilitator Superfamily, the ATP-Binding Cassette Superfamilies and the Resistance-Nodulation Cell Division[18]. The Resistance-Nodulation-cell Division (RND family) is responsible for macrolide intrinsic resistance in several Gram negative bacteria and it has been recently proposed also for *H. pylori*. In detail, it has been observed that 4 RND gene clusters (HP0605-HP0607, HP0971-HP0969, HP1327-HP1329, HP1489-HP1487) in the efflux pump system play a role in promoting multidrug *H. pylori* resistance[19]. These systems of excretion can be inhibited by the administration of specific Efflux Pump Inhibitors (EPI), such as Phe-Arg-β-naphthylamide (PAβN). Indeed, EPI-administration is associated with a relevant intracellular entrapment of antibiotic and a significant decrease of MIC values. In detail, a dose-dependent reduction of MIC values in 15 rRNA point mutate resistant strains has been demonstrated by using PAβN. Increased intracellular antibiotic concentrations able to compensate the reduced drug affinity for the mutate ribosomal site have been postulated as a possible mechanism. This effect is constantly associated with the HP0605-HP0607 cluster gene. Interestingly, a different effect of EPI administration on MIC values is observed between susceptible and rRNA mutate strains. A possible explanation is that, in susceptible strains, clarithromycin binds preferentially to the ribosomal subunits rather than the efflux pumps. Consequently, the excretive activity of efflux pumps becomes irrelevant, the effect of PAβN on MIC value modifications vanishing. On the contrary, in the rRNa mutate strains, clarithromycin is preferentially excreted by the efflux pumps because of its low affinity with the mutate ribosomal site, with the more relevant impact of efflux pumps inhibition by PAβN on MIC values[20]. Based on these findings, it is reasonable to hypothesize that PAβN (or PAβN-like molecules) administration could improve the eradicating efficacy of the clarithromycin-based therapies by increasing its intracellular entrapment.

The possible interaction between the RND efflux pump system and proton pump inhibitors (PPIs) due to structural analogies is also of clinical interest. Besides the deep gastric acid inhibition, PPIs may inhibit the activity of bacterial efflux pumps, similar to EPI drugs. Interestingly, MIC values of clarithromycin, as well as metronidazole, amoxicillin and furazolidone, are decreased 4-fold and 3-fold in *H. pylori* multi-resistant strains by using rabeprazole and pantoprazole respectively, whilst no significant effect is observed with omeprazole, esomeprazole and lansoprazole[21]. These differences should be considered when choosing the PPI in eradication regimens.

METRONIDAZOLE

Mechanisms of metronidazole resistance have been extensively investigated and new information has been recently obtained[22]. In *H. pylori* strains, MIC values of 0.5-2 mg/L are reported, antibiotic resistance being recognized with MIC values ⩾ 8 mg/L (range: 16-128 mg/L)[5,23]. Bactericide activity of metronidazole depends on the reduction

of its nitro-groups in anionic radicals, nitroso-derivates and hydroxylamines which are able to damage the DNA-helicoidal structure. These reactions of reduction are strongly dependent on the intracellular redox potential of components of electron transport chain which needs to be effectively negative. In detail, electrons are produced by the Pyruvate Oxido Reductase complex (POR) and are passed to either ferredoxin or flavodoxin which, in turn, reduce other molecules as metronidazole[24]. This process is particularly active in anaerobe species which are highly susceptible to metronidazole. The acquisition of antibiotic resistance depends on the reduction or abolition of activity of the electron carriers. On the contrary, the high intracellular redox potential of aerobe species prevents the metronidazole reduction-activation and is responsible for the intrinsic resistance of these bacteria[25].

A further action mechanism of metronidazole against anaerobe bacteria in aerobe conditions consists in the production of oxygen-free radicals. In this case, the oxygen acts as the last acceptor of electrons from reduced metronidazole, leading to the regeneration of the parent inactive antibiotic (futile cycle) and the production of oxygen-free radicals which are toxic for DNA structure[26]. In resistant strains, such a bactericide effect is neutralised by a catalase-superoxide dismutase system with final water production. This enzymatic system increases its activity in the presence of metronidazole[25,27]. The intracellular redox potential/oxygen tension also plays a relevant role in the resistance of microaerophilic species, such as *H. pylori*, in which catalase/superoxide dismutase is not present. Of note, the pre-exposure of *H. pylori* resistant strains to anaerobic conditions is associated with a loss of resistance and restoration of metronidazole susceptibility[28]. In this event, a NADH oxidase acts as an 'oxygen scavenger' assuring low redox potential/oxygen tension and maintaining the antibiotic in the active form. An inactive mutate NADH oxidase and intracellular higher redox potential/oxygen tension have been found in *H. pylori* resistant strains[29].

Different mutations involving the rdxA gene which encodes for an oxygen insensitive NADPH nitro-reductase have been identified in metronidazole resistant strains. These mutations are recognized as the main mechanism conferring metronidazole resistance in *H. pylori*[30]. In the susceptible strains, NADPH nitro-reductase reduces several compounds, including metronidazole, by 2 electrons transfer and generating toxic nitro-derivates for DNA. For example, the activation of NADPH in E. coli, which is usually resistant to metronidazole, generates susceptible strains. Besides these mutations, other and more complex genetic events (insertions and deletions of transposons, missense and frameshift mutations) could be simultaneously present in the rdxA gene. These events complicate metronidazole resistance assessment by bio-molecular tools[31-33].

More recently, the inactivation of other reductases, encoded by genes as frxA (for NADPH flavin oxidoreductase) and frxB (for ferredoxin-like enzymes), has been investigated. There is evidence that these point mutations are able to increase bacterial resistance exclusively in the presence of rdxA gene mutations[23,34-35]. Indeed, the rare cases of metronidazole resistant strains in the absence of rdxA mutations have been attributed to mutations involving genes outside the rdxA which can, in turn, down-modulate its expression[36].

A role for a complex efflux system responsible for metronidazole in *H. pylori* strains has been recently reported. In detail, the presence of Outer membrane Efflux Proteins (OEP) in *H. pylori* which interact with several intracellular translocases and regulate secretion of different antibiotics has been found. Of note, the inactivation of 2 OEPs (HP0605 and HP0971) in a double-knockout mutant strain significantly increased susceptibility towards metronidazole, supporting a significant role for this efflux pump system in metronidazole resistance[19].

LEVOFLOXACIN

The use of levofloxacin for *H. pylori* eradication is increasing worldwide because of its role in 'rescue therapy' regimens following the failure of clarithromycin-based treatments. MIC values of 0.25-0.50 mg/L are generally reported, antibiotic resistance being recognized with MIC values $\geqslant$ 1 mg/L (range: 4-32 mg/L)[37,38]. Fluoroquinolones exert a dose-dependent bactericide effect by binding the sub-unit A of DNA gyrase (topoisomerase Ⅱ), an essential enzyme for the maintenance of DNA helicoidal structure. In susceptible strains, levofloxacin stops DNA and, at high doses, even RNA synthesis. Surprisingly, when the dose is further increased, quinolones become bacteriostatic agents.

Point mutations in Quinolones Resistance-Determining Region (QRDR) of gyrA prevent binding between the antibiotic and the enzyme, conferring antibiotic bacterial resistance[39]. Different studies found the involvement of the following *H. pylori* loci: (1) position 91 (Asp91Gly, Asn, Ala, or Tyr); (2) position 87 (Asn87Lys); and (3) position 88 (Ala88Val)[39-41]. Mutations in both 91 and 87 position have been observed in the 100% of levofloxacin resistant isolates and a new mutation consisting in the substitution of Asn with Tyr in position 87 has been additionally identified[37]. Rare mutations involve the position 86 (Asp86Asn) which, in turn, is usually associated with the mutations at 87 and 91 positions[37], lowering its role on MIC values. Similarly, the constant association between the gyrB with the gyrA 87-91 mutations most likely minimize the role of the gyrB mutations in quinolone resistance[42]. Indeed, gyrA and gyrB gene mutations involvement in levofloxacin resistance has been observed in 83.8% and 4.4% respectively[43].

Other factors involved in levofloxacin resistance are an amino acidic polymorphism in the codon 87 of gyrA, consisting in the presence of different asparagyne-threonine residues. In detail, the complete sequencing genome of 2 strains, i.e. the 26695 and the J99, allowed identifying the presence of threonine in the J99 strain and asparagyne residues in the 26695 strain associated with a higher antibio-

tic susceptibility. Interestingly, the presence of threonine residue at 87 codon is also conserved in other. Helicobacter types thus indicating the possibility of a "philogenic" evolution of Helicobacter species[37].

AMOXICILLIN

Amoxicillin is a β-lactam antibiotic included in all current therapeutic regimens for *H. pylori* eradication[4]. MIC values ranging from 0.06 to 0.25 mg/L are generally reported in susceptible strains, antibiotic resistance being recognized with MIC values ⩾ 1 (range: 1-8 mg/L)[5,44]. Amoxicillin acts by interfering with the peptidoglycan synthesis, especially by blocking transporters named penicillin binding proteins (PBP)[5]. This drug has been the first antibiotic used in *H. pylori* therapy because of a presumed absence of resistance. Nevertheless, the evidence of stable amoxicillin resistant strains, with a MIC of 8 mg/L, has been reported[45]. Moreover, an instable amoxicillin resistance has been described in *H. pylori* isolates, the resistance being peculiarly lost upon freezing the culture at -80°C. Such an unusual condition has been defined as 'amoxicillin tolerance' rather than resistance[46].

Different mechanisms have been invoked in the stable amoxicillin resistance. The Penicillin Binding Proteins (PBPs) are enzymes involved in the synthesis of the peptidoglycan layer of the bacterial wall by a glycosyl transferase-acyl transpeptidase activity. This enzymatic activity is located in the C-terminal region, in 3 distinct motifs ($SKN_{368-371}$, $SNN_{433-435}$, $KTG_{555-557}$) of PBPs. The first motif occupies a central position in the catalytic cleft whereas second and third motifs are dislocated on the outside. PBP-1, PBP2, PBP3 are reported as high molecular PBPs whilst PBP4 is reported as low molecular protein. The β-lactam binding to PBPs motifs leads a bactericide effect by synthetic interruption of the peptidoglycan layer, as well as an osmotic bacterial shock. Production of β-lactamase, i. e. the main mechanism of penicillin resistance in other bacteria, has been consistently found to be inactive in *H. pylori*[47-49].

Several investigations indicate that multiple point mutations in pbp1 gene are the major mechanism of amoxicillin resistance, leading to a loss of affinity between amoxicillin and PBP-transpeptidase[44,50]. It has been observed that the Ser_{414} to Arg substitution, adjacent to the SKN motif in PBP1, is responsible for amoxicillin resistance with a significantly increased MIC (> 0.5-1 mg/L)[49]. Another study reported the substitution of Asn_{562} aminoacid with a Tyr residue in proximity to KTG motif of PBP1. Such a point mutation is able to confer high resistance to all strains *in vitro* and is considered the main mutation conferring resistance. Other substitutions (Ala_{369} to Thr, Val_{374} to Leu, Leu_{423} to Phe, Thr_{593} to Ala) not constantly associated with Asn_{562}-Tyr seem to play an additive role in increasing MIC values of the resistant strains similarly to point mutations in PBP2, in PBP3 and PBP4[48,51]. Interestingly, *H. pylori* resistant strains obtained by transformation *in vitro* of susceptible naive strains, exhibit MIC values 5-10 fold lower than naïve resistant strains[49], suggesting that several and concomitant mechanisms are probably involved in conferring the high levels observed in natural antibiotic resistance.

The outer bacterial membrane constitutes a first barrier for accounting for an intrinsic and not specific resistance. Indeed, the variable fluidity of lipopolysac caridic layer is able to limit the diffusion of several lipophilic compounds. Recent findings indicate that "porin" narrow channels, encoded in *H. pylori* by hopB and hopC genes, regulate the penetration of small solutes. Point mutations in hopB and a deletion in hopC gene are associated with reduced amoxicillin accumulation in all naïve mutant and transformed strains, with a consequent increase of MIC values (250 mg/L for hopB gene and 125 mg/L for hopC gene)[44]. When point mutations either in hopB or in hopC are associated with mutations in PBP1 gene (triple mutants), a further increase of MIC values (400 mg/L) is observed. These findings could suggest that channels and PBP1 mutations are factors able to support the resistance[44,52]. It has been reported that several encoding "porin" genes could be over-regulated (omp25 porin gene) or down-regulated (omp32 porin gene) by antibiotic exposure leading to alterations in the membrane permeability. Comparable alterations of permeability are likely associated to variable expression of genes involved in import/export/binding of metals[53].

Finally, the efflux of molecules is a frequently reported event in bacteria as a protective process from the toxic effect of environmental compound accumulation. Nevertheless, it seems unlikely that amoxicillin resistance is sustained by these mechanisms because amoxicillin shows a very low hydrophobicity which is an indispensable requirement for substrates of multidrug efflux pumps[54,55].

TETRACYCLINE

Tetracycline is a fundamental antibiotic in quadruple regimens for *H. pylori* eradication. MIC values 0.25-2 mg/L[56] are generally reported, antibiotic resistance being recognized with MIC values ⩾ 4[5]. Bacterial resistance towards such a drug, although still rare, appears to be increasing. Tetracycline acts as a bacteriostatic against either Gram positive or Gram negative species by inhibiting codon-anticodon link at level of 30S ribosomal subunit and blocking the attachment of aminoacyl-tRNA to the acceptor site. Resistant strains show wide range of MIC values (2-256 mg/L). Recent studies have identified 2-6 possible sites for antibiotic-ribosome interaction at high affinity, whilst several biochemical investigations reported multiple, likely hundreds, sites at low affinity[57,58]. Simultaneous triple point mutations from the 965 to 967 position in loop of helix 31 - i.e. the crucial part of primary acceptor site (site P) is recognized as the major mechanism of tetracycline resistance. The main point mutation is a substitution of an AGA with a TTC triplet[59,60] and it reduces the affinity of 24%-52%[61]. Levels of resistance are proportional to the number of changes in the AGA 965-967. Single and double point mutations are associated with low and inter-

Table 1 Minimal inhibitory concentrations of the different antibiotics (left side) and main mechanisms of resistance induction for each antibiotic (right side)

Antibiotics	MIC in susceptible strains	MIC in resistant strains	Mechanisms of resistance
Clarithromycin	0.016-0.50 mg/L	⩾ 1 mg/L (2-256 mg/L)	Point mutations in rRNA Efflux pumps system (RND family)
Metronidazole	0.5-2 mg/L	⩾ 8 mg/L (16-128 mg/L)	Mutate NADPH reductase Mutate NADH oxidase Other efflux pumps
Levoxacin	0.25-0.50 mg/L	⩾ 1 mg/L (4-32 mg/L)	Point mutations in QRDR of gyrA gene Polymorphism in 87 codon of gyrA
Amoxicillin	0.06-0.25 mg/L	⩾ 1.0mg/L (1-8 mg/L)	Point mutations in pbp genes Point mutations in hpB and hpC genes Point mutations in omp25 and omp32 porin genes
Tetracycline	0.25- 2 mg/L	⩾ 4 mg/L (2-256 mg/L)	Point mutations in primary binding Tet-P site Point mutations in Tet-4 secondary inding site Alterations by oxidoreductase

MIC: Minimal inhibitory concentrations.

mediate MIC values whilst high resistance levels are observed in the presence of a triple mutation from AGA 956 to 957. In detail, among the possible mutations in AGA triplet, the substitution involving the Guanine in the central position is associated with higher MIC values, suggesting that purine base plays a more consistent role in the configuration of the primary site. Purine-rich sequences in the loop of helix 31 are more frequently observed in susceptible strains, whilst pyrimidine-rich loops are in the resistant strains. It is possible that pyrimidine-rich sequences in helix 31 are not compatible with tetracycline conformation, leading to a decreasing affinity[60]. Another study found a deletion of G942 in all resistant strains. This guanine base is located in Tet-4 site, in proximity of primary *P* site. Since the affinity of tetracycline for Tet-4 site is significantly lower than those for primary *P* site, Tet-4 may be considered an accessory site for the antibiotic activity in susceptible strains. Therefore, the loss of affinity due to a deletion G492 in such a site may exert a marginal role in the increasing bacterial resistance[62] (Table 1).

Serial exposures of susceptible strains on antibiotic are unable to confer resistance whereas the exposition to mutate resistant DNA leads easily transformation. These data indicate a horizontal spread of mutate genome rather than a vertical or parental transformation[62]. Of note, resistant transformants from susceptible strains exhibit intermediate MIC values between parental susceptible strains (4-8 mg/dL) and natural resistant strains (> 32 mg/dL)[61,63]. Such a finding would indicate that factors other than point mutations in 30S ribosomal subunit may work in concert for the tetracycline resistance development. Indeed, resistant strains without point mutations have been observed[61].

Another mechanism of tetracycline resistance is attributed to ribosomal protection by the soluble protein Tet (O). Such a protein removes the antibiotic from ribosome preventing the arrest of protein synthesis[64]. In addition a chemical modification of tetracycline by an oxidoreductase NADP-dependent may interfere in the binding between antibiotic and the ribosomal site[58].

Decreased membrane permeability and a reduced intracellular accumulation of tetracycline were observed in tetracycline resistant strains, which are also cross-resistant to amoxicillin. This finding suggests an identical profile of outer protein for both antibiotics. Finally, the possible role of a specific tetracycline efflux pumps system affecting intracellular drug concentrations has been investigated with discordant results. Indeed, pre-exposure of resistant strains to a de-energizing agent such as cyanide m-chlorophenylhydrazone (CCCP) leads to variable reductions of MIC values[21,58]. However, the role of either specific pumps unaffected by CCCP or a variable expression of not specific multidrug efflux pumps, such as the MexAB-OprM system, cannot be excluded and should be further investigated[62,63].

CONCLUSION

The amount of data we have reported in this editorial reveals that the knowledge about *H. pylori* antibiotic resistance is a topic with a rapidly and constantly increasing interest. Future perspectives hope for new information aimed at elaborating novel and rational antibiotic associations very effective for *H. pylori* infection cure in clinical practice. Another "fascinating challenge" could be a feasible, cheap and not time consuming laboratory investigation able to predict the treatment outcome and address the best therapeutic choice case by case.

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Non-erosive and uncomplicated erosive reflux diseases: Difference in physiopathological and symptom pattern

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Abstract

AIM: To investigate differences in the physiopathological findings (manometry and pH monitoring) and symptoms between cases of non-erosive reflux disease (NERD) and erosive reflux disease (ERD) found positive at 24 h pH monitoring.

METHODS: For a total of 670 patients who underwent 24 h pH monitoring, esophageal manometry and upper endoscopy were retrospectively evaluated, assessing the reflux symptoms, manometric characteristics of the lower esophageal sphincter (LES) and esophageal body and the presence or absence of esophagitis and hiatal hernia. Typical and atypical symptoms were also evaluated. For inclusion in the study, patients had to have NERD or ERD and be found positive on pH monitoring (NERD+). Patients with Gastroesophageal reflux disease (GERD) complicated by stenosis, ulcers or Barrett's esophagus were ruled out.

RESULTS: 214 patients were involved in the study, i.e. 107 cases of NERD+ and 107 of ERD. There were no significant gender- or age-related differences between the two groups. The ERD group had more cases of hiatal hernia (P = 0.02) and more acid reflux, both in terms of number of reflux episodes (P = 0.01) and as a percentage of the total time with a pH < 4 (P = 0.00), when upright (P = 0.007) and supine (P = 0.00). The NERD+ cases had more reflux episodes while upright (P = 0.02) and the ERD cases while supine (P = 0.01). The LES pressure was higher in cases of NERD+ (P = 0.03) while the amplitude and duration of their esophageal peristaltic waves tended to be better than in the ERD group (P >0.05). The NERD+ patients presented more often with atypical symptoms (P = 0.01).

CONCLUSION: The NERD+ patients' fewer reflux episodes and the fact that they occurred mainly while in the upright position (unlike the cases of ERD) may be two factors that do not favor the onset of esophagitis. The frequently atypical symptoms seen in patients with NERD+ need to be accurately evaluated for therapeutic purposes because patients with GERD and atypical symptoms generally respond only partially to medical and surgical treatments.

Key words: Gastroesophageal reflux disease; Non-erosive reflux disease; Erosive reflux disease; Barrett's esophagus; Reflux symptoms

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INTRODUCTION

Gastroesophageal reflux disease (GERD) develops when the contents of the stomach flow back into the esophagus, causing troublesome symptoms and sometimes damaging the mucosa and leading to complications[1]. This definition actually covers different conditions, ranging from non-erosive reflux disease (NERD) to erosive reflux disease (ERD), to forms of GERD complicated by ulcers or stenosis and Barrett's esophagus (BE). These various forms of GERD are often interpreted on the strength of a "spectrum model", on the assumption that the disease progresses in steps to a more severe form and may occasionally regress to a less severe form. NERD, ERD and BE are thus usually configured as different stages of the same disease[2,3]. On the other hand, these features of GERD are sometimes seen as three different categories of patients in which it is rare to see a patient's transition from one group to another[4,5]. From the clinical standpoint, 50%-75% of individuals with GERD have an intact esophageal mucosa[4,6]. These NERD patients are also the cases that respond the least and the least predictably to medical therapy[7]. In recent years, various researchers have attempted to characterize the various ways in which GERD can become manifest from the physiopathological, symptomatic and anatomopathological standpoints, often with contradictory results[8].

The aim of this study was to investigate a cohort of patients with NERD found positive on pH monitoring (NERD+) as compared with a cohort of patients with uncomplicated erosive reflux disease and ascertained ERD. These two groups of patients can be seen as closely juxtaposed in the "spectrum" model but very different from the categorial standpoint. Our main aim was to evaluate whether differences exist between NERD+ and ERD patients in terms of their manometric variables detectable in the lower esophageal sphincter (LES) and esophageal body and the outcome of their pH monitoring. The second endpoint of this study was to see whether there was any difference between the two groups in the clinical presentation of their symptoms.

MATERIALS AND METHODS

Type of study

We performed a retrospective clinical assessment.

Study population and inclusion criteria

We considered all the reports on patients referred to the digestive physiopathology laboratory at the Surgery Department at the University of Udine from 1998 to 2010 who underwent esophageal manometry and 24 h pH monitoring.

To be included in the study, patients had to fulfill the following criteria:

They were positive on 24 h pH monitoring (DeMeester score > 14.8) and consequently diagnosed with GERD: pH monitoring was done with a pH catheter with an antimonium electrode (Zinecties 24; Medtronic) positioned 5 cm from the upper margin of the LES (previously identified by manometry). The data collected over 24 h were recorded in a portable data logger (Digitrapper MkIII, Synecties Medical) and subsequently processed using the manufacturer's software.

They had undergone esophageal manometry: Stationary esophageal manometry was completed before pH monitoring using an 8 channel catheter perfused with water to establish the site and features of the LES. Peristalsis and the related pressures on a level with the esophageal body were assessed in 10 wet swallows. All data were processed using the Polygram for Windows software by Medtronic.

They had undergone esophagogastroduodenoscopy: NERD+ patients were not to have been taking any medical therapy prior to the test.

ERD patients' esophagitis had to be classifiable as grade 1-2 according to the Los Angeles classification: Severe or complicated esophagitis or Barrett's esophagus were considered exclusion criteria.

Data collection

Data were collected on the following:

Demographic: Gender and age.

Endoscopic: Presence/absence of uncomplicated esophagitis (ERD/NERD) and hiatal hernia (judged to be present when the distance between the diaphragmatic pinchcock and the gastro esophageal junction was > 2 cm).

Manometric: Mean pressure, total and abdominal lengths of the LES; mean proximal and distal wave amplitude and duration in the esophageal body; effective of peristalsis (i.e. absence of specific motor anomalies or aspecific motor disorders, defined as peristaltic waves with an amplitude < 30 mmHg on a level with the distal esophagus or pathological waves with no contractions or with double or triple peaks in > 30% of 10 wet swallows during manometry of the esophageal body).

pH monitoring: Total number of reflux episodes, number of reflux episodes persisting more than 5 min, percentage of the total time with pH < 4, in a supine or upright position, DeMeester score.

Table 1 Description of the study population *n*(%)

Population	NERD+	ERD	*P* value
Gender			
Males	39 (36.5)	35 (32.8)	NS
Females	68 (63.5)	72 (67.2)	
Age			
mean ± SD	52.08 ± 13	52.8 ± 14	NS
Median	55	54	
Hiatal hernia	53 (43.4)	69 (56.6)	0.02

NERD+: Non-erosive reflux disease positive on pH monitoring; ERD: Erosive reflux disease; NS: Non-statistically significative.

Symptoms: Symptoms were reported as typical (heartburn, regurgitation) or atypical (respiratory, otorhinolaryngological, cardiac symptoms).

Statistical analysis

The data were described using means, medians and standard deviations (SD). The frequencies were also described using percentages where applicable. Continuous variables were compared using Student's *t*-test for data with a normal distribution and the Mann-Whitney test in the remaining cases. Proportions were compared using the chi square test. Odds ratios were calculated for 95% confidence intervals. The value of the single tests was considered significant where $P < 0.05$. The data analysis was conducted using the SPSS, rel. 18 (Chicago, IL, USA).

RESULTS

Study population

From 1998 to 2010, a total of 670 patients were assessed at our surgical physiopathology laboratory; 214 of them met all the previously-stated inclusion criteria and were included in the study. There were 107 (50%) cases with evidence of esophagitis (ERD) and 107 (50%) with no esophageal lesions (NERD+).

Demographic characteristics

Of the 214 patients considered, 74 (34.6%) were female and 140 (65.4%) were male. In the two patient groups, NERD+ and ERD, there were 39/107 (36.5%) and 35/107 (32.8%) women respectively and 68/107 (63.5%) and 72/107 (67.2%) men. The mean age of the study population was 52.49 ± 14 years (median 54.50); it was 52.08 ± 13 (median 55) in the NERD+ group and 52.8 ± 14 (median 54) in the ERD group.

The two groups were judged to be homogeneous for both the demographic variables considered ($P < 0.05$)(Table 1).

Hiatal hernia

Hiatal hernia was found in 122 (57%) patients, i.e. in 53 cases of NERD+ (43.4%) and 69 cases of ERD (56.6%). The cases of ERD were therefore more frequently associated with hiatal hernia ($P = 0.02$).

Physiopathological patterns

Esophageal body manometric characteristics: The peristalsis assessment in the study population as a whole identified distal waves with a mean amplitude of 77.97 ± 42 mmHg and a mean duration of 3.61 ± 0.9 s; at proximal level, the mean values were 50.68 ± 21 mmHg and 2.87 ± 0.6 s respectively. In the NERD+ group, the mean wave amplitude was 80.38 ± 45 at distal level and 50.61 ± 20 mmHg at proximal level, while the waves' duration was 3.58 ± 1 and 2.83 ± 1 s respectively. In the ERD group, the mean distal and proximal wave amplitude was 75.55 ± 40 and 50.74 ± 21.mmHg respectively and their duration was 3.64 ± 0.9 and 2.92 ± 0.6 s.

No significant differences emerged on comparing the amplitude and duration of the distal and proximal peristaltic waves. The two groups of patients were also similar as regards the efficacy of peristalsis variable, i.e. 41 NERD+ patients (47.7%) and 45 ERD patients (52.3%) had an ineffectual peristalsis (P = NS).

Lower esophageal sphincter manometric characteristics: For the study population as a whole, manometry of the LES identified a mean pressure of 10.33 ± 6 mmHg, a mean total length of the LES of 2.51 ± 0.8 cm and a mean abdominal length of 1.09 ± 0.9 cm. In the two patient groups, NERD+ and ERD, the mean values were respectively: 11.18 ± 6.5 and 9.4 ± 5.3 mmHg for the pressure; 2.57 ± 0.7 and 2.44 ± 0.8 cm for the total length of the LES; and 1.13 ± 0.8 and 1.05 ± 0.9 cm for its abdominal length.

NERD+ patients had a significantly more severe pressure insufficiency than ERD patients ($P = 0.037$). On the other hand, the length of the LES was not dissimilar in the two groups ($P < 0.05$) (Table 2).

pH monitoring characteristics: For the study population as a whole, the mean number of reflux episodes was 144.3 ± 106.2 and the episodes lasting > 5 min amounted to a mean 6.3 ± 6.78. The NERD+ patients had a mean 125.67 ± 74.49 reflux episodes and those lasting > 5 min amounted to a mean 4.42 ± 4.9. In the ERD group, the figures were 162.93 ± 128.15 and 8.19 ± 7.78 respectively so these patients with endoscopic findings positive for esophagitis had significantly more and more persistent reflux episodes than the patients without esophagitis (respectively $P = 0.01$; $P = 0.00$). In the study population as a whole, we recorded a total percentage of the time with a pH < 4 of 12.5 ± 11.8, with 12.2 ± 10.9 for the upright position and 12.9 ± 17.7 for the supine position. In the NERD+ group, the percentage of the total time with a pH < 4 and the corresponding percentages for the upright and supine positions were respectively: 9.24 ± 8.1, 10.2 ± 7.9 and 7.6 ± 12.2. In the ERD group, the three values were: 15.8 ± 14, 14.2 ± 13 and 18.2 ± 20.6. The proportion of time with a pH < 4 was significantly higher in the ERD group than in the NERD+ group, both for the period as a whole ($P = 0.000$) and after distinguishing between the two positions, upright ($P = 0.007$) and supine ($P = 0.000$).

Table 2 NERD+ *vs* ERD: manometry study

Manometry	NERD+	ERD	*P* value
LES			
Pressure (mmHg)			
mean ± SD	11.18 ± 6.5	9.4 ± 5.3	0.03
Median	9.33	8.5	
Total length (cm)			
mean ± SD	2.57 ± 0.7	2.44 ± 0.8	NS
Median	3	3	
Abdominal length (cm)			
mean ± SD	1.13 ± 0.8	1.05 ± 0.9	NS
Median	1	1	
Esophageal body			
Distal wave amplitude (mmHg)			
mean ± SD	80.38 ± 45	75.55 ± 40	NS
Median	70.2	68.3	
Proximal wave amplitude (mmHg)			
mean ± SD	50.61 ± 20	50.74 ± 21	NS
Median	50	48.65	
Distal wave duration (s)			
mean ± SD	3.58 ± 1	3.64 ± 0.9	NS
Median	3.45	3.4	
Proximal wave duration (s)			
mean ± SD	2.83 ± 1	2.92 ± 0.6	NS
Median	2.75	2.75	
Effective peristalsis (%)	41 (47.7%)	45 (52.3%)	NS

LES: Lower esophageal sphincter; NERD+: Non-erosive reflux disease positive on pH monitoring; ERD: Erosive reflux disease; NS: Non-statistically significative.

Table 3 NERD+ *vs* ERD: pH monitoring

24 h pH monitoring	NERD+	ERD	*P* value
Number of reflux episodes			
Total			
mean ± SD	125.67 ± 74.49	162.93 ± 128.1	0.01
Median	118	131	
Lasting > 5 min			
mean ± SD	4.42 ± 4.9	8.19 ± 7.7	0
Median	3	6	
pH < 4			
Total time (%)			
mean ± SD	9.24 ± 8.1	15.80 ± 14	0
Median	6.8	11	
Upright time (%)			
mean ± SD	10.20 ± 7.9[a]	14.20 ± 13[b]	0.007
Median	7.8	10.7	
Supine time (%)			
mean ± SD	7.6 ± 12.2[a]	18.20 ± 20.6[b]	0
Median	4.1	11.1	
DeMeester score			
mean ± SD	37.24 ± 32.63	63.38 ± 48.70	0
Median	30.4	48.7	

NERD+: Non-erosive reflux disease positive on pH monitoring; ERD: Erosive reflux disease; NS: Non-statistically significative; [a]NERD+: Upright *vs* supine ($P = 0.02$); [b]ERD Supine *vs* upright ($P = 0.01$).

Table 4 Clinical presentation of NERD+ and ERD patients *n*(%)

Clinical presentation	NERD+ (88/107)	ERD (102/107)	*P* value
Typical symptoms only	52 (59.1)	76 (74.5)	0.01
Typical and atypical symptoms	36 (40.9)	26 (25.5)	0.01

NERD+: Non erosive reflux disease positive on pH monitoring; ERD: Erosive reflux disease.

In the ERD patients, the percentage of the time with a pH < 4 was longer in the supine position ($P = 0.01$) than when upright; vice versa, in the NERD+ patients reflux was more prevalent when patients were upright ($P = 0.024$).

The analysis of the DeMeester scores indicated a mean value for the total population of 50.3 ± 43.38. For the NERD+ patients, the mean DeMeester score was 37.24 ± 32.63 while the ERD patients had a significantly higher mean score of 63.38 ± 48.70 ($P = 0.00$)(Table 3).

Symptom pattern: On the whole, 128 patients (67.4%) had typical esophageal symptoms while 62 (32.6%) reported typical and atypical, or only atypical symptoms. No data regarding symptoms were available in the clinical records of 24 patients (11.2%; 5 ERD and 19 NERD+) so these cases were not considered for this parameter. In the NERD+ group, 52/88 patients (59.1%) had typical symptoms while the other 36 (40.9%) had a typical and atypical, or entirely atypical symptom pattern. Conversely, the patients with typical symptoms in the ERD group amounted to 76 (74.5%) while 26 (25.5%) reported atypical symptoms. Patients with esophagitis thus presented a typical symptom pattern far more frequently than those with NERD+ ($P = 0.01$). The latter have a high probability of developing atypical symptoms with an odds ratio of 2.02 (95% CI, 1.05-3.93)(Table 4).

DISCUSSION

General considerations: study population

The noteworthy feature of this study lies in that we configured two groups of patients, each of which was particularly homogeneous. The NERD+ group only included patients with ascertained pathological reflux, disregarding any cases with symptoms but no confirmed pathological reflux (NERD-), which are associated with a hypersensitive esophagus, functional heartburn or non-acid reflux[9,10], all controversial physiopathological explanations that are often difficult to demonstrate[8]. Assessing heterogeneous groups of NERD patients (NERD+ and NERD-) or with a diagnosis of NERD based exclusively on symptoms and endoscopic evidence (as some researchers have done) can trigger a cascade of biases affecting the interpretation of the results[11]. Bearing this in mind, our study has one of the most numerous cohorts of NERD+ cases to have been investigated in the literature from the physiopathological standpoint.

Another important aspect of our study lies in the lack of any differences between our two groups of patients regarding their demographic characteristics. In particular, the fact that the mean age of the ERD group was similar to that of the NERD+ patients (around 50 years old in both cases) seems to contradict the claim that patients with-

out esophagitis tend to be younger and can be expected to progress towards a picture of full-blown esophagitis as they grow older[12,13]. Other authors reported individuals with complicated reflux disease being older than patients with NERD+, although the latter were actually much the same age as the group with ERD, as in our population[14].

We also found no gender-related differences in our two patient groups despite the literature reporting a tendency to find more females among NERD patients than among cases of esophagitis[12,15,16].

In our study population, the presence of hiatal hernia was associated more frequently with a picture of reflux with esophagitis. Hiatal hernia is currently assumed to be one of the physiopathological factors contributing to the onset of GERD by reducing LES competence and interfering with esophageal clearance[17,18]. Hiatal hernia is apparently a dominant predictor of erosive esophagitis[19] but it has been little studied in patients with NERD who are less likely to have hiatal hernias than patients with esophagitis[14,5,20].

Physiopathological pattern

Resting LES pressure was found to be higher in our patients with NERD+ while the other two sphincter competence variables considered (total and abdominal length) were similar in the two groups. A tendency for NERD+ patients to have a higher mean LES pressure than ERD patients has also been reported in other studies, although the difference failed to reach statistical significance[14,20].

Findings for esophageal body motility did not differ significantly between patients with NERD+ and those with ERD, apart from a slight tendency for the distal wave amplitude to be greater in the first group. Much the same can be said of the efficacy of peristalsis since the NERD+ patients tended less to have an ineffective peristalsis. If we consider distal wave amplitude and efficacy of peristalsis as important parameters in the process of esophageal clearance, we could say that NERD+ patients tend to have a better esophageal clearance but not to any significant degree. That NERD+ patients have distal esophageal waves with a higher mean amplitude than patients with esophagitis has also been reported by other researchers[14].

NERD+ patients were found to have less severe reflux than ERD patients in terms of both the total number of reflux episodes and the percentage of the time with a pH < 4 in the upright position and when supine at night; this situation was confirmed by the former having a lower DeMeester score. Patients with NERD+ have more reflux when upright in the daytime than at night whereas reflux is more common at night in the group with esophagitis; this finding is certainly worth noting because night-time reflux is known to be more harmful to the esophageal mucosa[21]. It would therefore seem from this study that, in addition to NERD+ and ERD patients experiencing a different number of reflux episodes, the timing of their reflux episodes is also different (when upright during the day or supine at night) and this could explain the presence or absence of lesions affecting the esophageal mucosa. In the few studies conducted on this issue, findings have been contradictory and often supported by a small number of patients.

The results of our study are consistent with Frazzoni's demonstration of a higher percentage of total and night-time reflux in ERD than in NERD patients. That a different reflux pattern exists between NERD and ERD has also been suggested in other studies[22,23]. On the other hand, a study conducted by Martinez (on 36 ERD patients and 71 NERD patients, the latter including cases both positive and negative on pH monitoring) reported NERD having a lower acid exposure, but with the 39 NERD- patients we disregarded, the 32 NERD+ patients no longer differed from those with ERD[24]. Unlike the situation seen in our study, excluding patients with functional heartburn seems to make the NERD+ and ERD cases overlap in terms of severity of acid reflux[15,25,26]. In NERD patients, therefore, the progression towards esophagitis might correlate more with the duration of their disease than with any greater quantity of acid reflux[27].

Symptom patterns

There is no evidence in the literature of symptom patterns (nature and severity of the symptoms) differing between cases of ERD and NERD when the latter types of patient include NERD+ and NERD- cases[16].

In our study, however, the clinical presentation of patients with NERD+ differed significantly from those with esophagitis, i.e. the former presented more frequently with atypical symptoms. Quantitative differences in patients' reflux episodes might be seen to support the "spectrum model", based on the assumption that NERD+ patients will become ERD cases with time due to their reflux episodes increasing secondary, for instance, to a further impairment of LES competence and esophageal clearance. Conversely, the different prevalent symptom patterns in the two populations (typical symptoms in ERD and atypical symptoms in NERD+) seem instead to support the categorial view[28] since it is difficult to imagine patients with mainly atypical symptoms progressing with time towards a different symptom pattern in which typical symptoms prevail.

In conclusion, our study demonstrates that NERD+ patients are not very dissimilar from cases of ERD from the functional standpoint, despite a lower acid exposure, a better sphincter competence and a tendency to have a better esophageal clearance. On the other hand, the two patient groups reveal a different prevalence of symptoms, more typical in ERD and atypical in NERD+. For the latter patients, pH monitoring plays a fundamental part in distinguishing patients with a normal acid exposure (NERD- from those with an abnormal contact time (NERD+). This is fundamental, particularly when dealing with NERD patients failing to respond to medical therapy with proton pump inhibitors (PPI) or when considering surgery for such patients[29]. In fact, NERD+ patients respond better than NERD- to medical therapy with standard-dose PPI[30] while the finding of a pathological reflux on pH monitoring in patients with reflux symptoms is a positive predictor of the success of surgery[31].

As regards symptoms, patients with NERD+ can be further divided into two subpopulations with or without atypical symptoms. NERD+ patients with atypical symptoms are more difficult to treat from both the medical and the surgical standpoint, being those least responsive to treatment with PPI or surgical anti-reflux procedures[32,33,34].

COMMENTS

Background

Gastro esophageal reflux disease (GERD) has approached as a spectrum of disease, ranging from non-erosive reflux disease (NERD) to erosive reflux disease (ERD), to form of GERD complicated. In literature NERD population is poorly defined as for physiopathology and related symptoms. The current article investigates physiopathological findings and symptoms between NERD and ERD population

Research frontiers

Important areas in these fields are to better understand which are the best diagnostic studies and the best therapeutic treatments in NERD patients with atypical symptoms.

Innovations and breakthroughs

The physiopathological study in the NERD population with reflux showed a different outcome if compared to ERD population; in the first group, the reflux episodes are fewer and occurred mainly in upright position. Moreover NERD patients have frequently atypical symptoms.

Applications

This study demonstrates that in the clinical practice, patients without esophagitis but with ERD symptoms could benefit of the physiopathological study for a better definition of their disease.

Peer review

The study provides evidences that the NERD+ patients have fewer reflux episodes and frequently atypical symptoms compared with ERD patients. It is good for the readership of this journal, especially for the gastroenterologist and GERD patients, even relevant for the normal population.

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CASE REPORT

Resolution of metabolic syndrome after following a gluten free diet in an adult woman diagnosed with celiac disease

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Abstract

Adult celiac disease (CD) presents with very diverse symptoms that are clearly different from those typically seen in pediatric patients, including ferropenic anemia, dyspepsia, endocrine alterations and elevated transaminase concentration. We present the case of a 51-year-old overweight woman with altered basal blood glucose, hypercholesterolemia, hypertriglyceridemia and persisting elevated transaminase levels, who showed all the symptoms for a diagnosis of metabolic syndrome. Because she presented iron deficiency anemia, she was referred to the gastroenterology department and subsequently diagnosed with celiac disease after duodenal biopsies and detection of a compatible HLA haplotype. Gluten-free diet (GFD) was prescribed and after 6 mo the patient showed resolution of laboratory abnormalities (including recovering anemia and iron reserves, normalization of altered lipid and liver function parameters and decrease of glucose blood levels). No changes in weight or waist circumference were observed and no significant changes in diet were documented apart from the GFD. The present case study is the first reported description of an association between CD and metabolic syndrome, and invites investigation of the metabolic changes induced by gluten in celiac patients.

Key words: Celiac disease; Metabolic syndrome; Gluten-free diet

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INTRODUCTION

Celiac disease (CD) is a systemic disorder characterized by enteropathy secondary to an altered immune response induced and maintained by exposure to gluten in the diet. CD is observed in genetically susceptible individuals and is one of the main causes of malabsorption in Europe[1]. The pathogenesis of the disease includes the activation of cytotoxic T lymphocytes in the lamina propria of the duodenum, together with the production of autoantibodies, giving rise to duodenal mucosal lesions of variable intensity as well as manifestations in other organs[2]. Approximately 1% of the world population is affected, and many patients go undiagnosed for years. Over half of all new cases are diag-

Table 1 Laboratory test parameters of the patient before and after 6 mo following a gluten free diet

Test parameter	Normal value	Before GFD	After GFD
Glucose	70-110 mg/dL	124.00	92.00
Cholesterol	140-200 mg/dL	338.00	212.00
Triglycerides	45-150 mg/dL	229.00	52.00
CRP	0-0.5 mg/dL	2.00	0.60
AP	40-105 IU/L	50.00	52.00
AST	10-40 IU/L	88.00	17.00
ALT	10-40 IU/L	82.00	18.00
GGT	10-40 IU/L	372.00	24.00
Hemoglobin	12-16 g/dL	10.80	12.60
MCV	80-100 fL	85.40	95.30
Ferritin	30-284 ng/mL	12.00	27.00
Vit. B12	197-866 pg/mL	598.00	817.00
FA	3-17.5 ng/mL	14.30	14.90
25OH-Vit. D^3	>40 ng/mL	23.00	39.00
TSH	0.27-4.24 μIU/mL	3.24	2.60
Free T4	0.93-1.78 ng/dL	1.01	1.05

GFD: Gluten free diet; CRP: C-reactive protein; AP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase; MCV: Mean corpuscular volume; AF: Folic acid; 25OH-Vit. D^3: calcidiol; TSH: thyroid stimulating hormone; Free T4: Free thyroxine.

nosed in adults[3]. Indeed, CD can by no means be considered as a childhood disease, and it has been estimated that 20% of all patients are over the age of 60 at the time of the diagnosis[4]. Adult CD manifests with very diverse symptoms that are clearly different from those typically seen in pediatric patients. Particular symptoms include iron deficiency anemia, dyspepsia, endocrine alterations (particularly of the thyroid gland), neuropsychiatric manifestations, osteoporosis and infertility. At the time of diagnosis, over half of all celiac patients present with chronic constipation rather than diarrhea. As a result of this varied range of symptoms, the estimated delay in diagnosing the adult disease is 11 years from the onset of symptoms[2]. This makes it necessary to maintain an active search for new cases, since it has been estimated that there are 2-7 undetected cases for every known patient with the disease[4]. As can be seen from the case described below, CD is also able to simulate disorders characteristic of metabolic syndrome, resulting in delayed diagnosis and treatment of the disease. In this context, effective treatment consists of a gluten free diet (GFD).

CASE REPORT

A 51-year-old woman was referred to the endocrinologist due to hypercholesterolemia. Her history reflected depressive syndrome treated with duloxetine, chronic constipation and dyspepsia, for which rabeprazole had been prescribed (20 mg/d). Body weight was 75 kg, with a body mass index (BMI) of 28.22 kg/m^2, and a waist circumference (WC) of 93 cm. The laboratory tests revealed normocytic anemia, altered basal blood glucose, hypercholesterolemia, hypertriglyceridemia and elevated transaminase levels-the latter having been detected in earlier tests (Table 1). On the basis of these findings, written instructions were provided for hyperlipidemia control without caloric restrictions and the patient was referred to the Department of Gastroenterology where studies were made to establish the origin of the altered liver biochemical profile, the anemia and symptoms of dyspepsia. The serology findings for hepatotropic viruses proved negative, and the iron and copper metabolic results were normal. There was no alpha-1-antitrypsin deficit, and no thyroid gland alterations were detected. The immunoglobulins were within normal limits, and the non-organ specific antibody titers proved negative. The tissue anti-transglutaminase IgA levels were within the normal range (1.1 IU/mL), and the hepatobiliary ultrasound findings were normal. Gastroscopy and colonoscopy likewise revealed no alterations, and multiple biopsies were obtained from the gastric antrum and second portion of the duodenum. The histological study revealed a dense intraepithelial lymphocyte infiltration in the duodenal mucosa (> 40%, mostly CD3+/CD8+ after immunohistochemical staining), consistent with lymphocytic enteritis or grade 1 of the classification developed by Marsh for CD[5]. The gastric mucosa was normal, with no evidence of *Helicobacter pylori*. A genetic study, made at this point, confirmed the presence of HLA-DQ2, a risk genotype for CD. As a result, CD was diagnosed and specific management was started in the form of GFD.

Six months later, the patient had correctly followed the prescribed GFD, but claimed to have failed to adhere to the low-fat recommendations, due to the restrictions to which she was already subjected. A diet questionnaire was administered by qualified personnel, identifying no changes in total intake or in macronutrient distribution. The patient consumed hardly any manufactured products, refined sugars or fried foods before starting GFD and,as a result, there were no significant changes following introduction of the latter diet. No proprietary and independent cause of CD was identified which could explain the laboratory test changes.The patient's constipation and mood state had improved. A slight weight loss was observed (73 kg, BMI 27.5 kg/m^2), with no significant changes in WC as measured by the same observer (92 cm). The laboratory tests showed resolution of the anemia and recovery of her iron reserves, with normalization of the transaminase levels and a decrease in cholesterol and triglycerides (37% and 77%, respectively) (Table 1), indicating that the metabolic disorders were practically resolved.

DISCUSSION

The present case study is the first description of an association between CD and metabolic syndrome, with reversion following the prescription of a GFD, together with complete resolution of ferropenic anemia and elevated transaminase levels. The clinical characteristics of the patient (altered lipid and liver function parameters) were consistent with metabolic syndrome, a condition which following its initial description by Reaven in 1988[6] has received nume-

rous definitions in seeking a central reference parameter (e.g. insulin resistance or abdominal obesity) capable of confirming its existence as a single clinical entity. However, the many definitions and lack of uniformity have led even Reaven to deny the existence of metabolic syndrome as a distinct clinical entity, thereby stressing the importance of treating rather than of defining the condition. The present case shows how adult CD can present as metabolic syndrome according to any of its definitions[7], despite the fact that CD is presently not included in the differential diagnosis of hyperlipidemia and has not been given importance in the context of metabolic syndrome.

In contrast, the association of CD with endocrine and metabolic alterations has been known for a long time. Type 1 diabetes mellitus classically has been associated with CD, and the possible corrective role of GFD upon the metabolic alterations of the disease has been extensively studied. When both disorders coexist, the introduction of GFD results in fewer hypoglycemic episodes, with no changes in glycosylated haemoglobin values[8]. A study in type 1 diabetic children diagnosed with CD demonstrated a recovery of normal BMI after following a GFD, together with improvement in glycosylated haemoglobin values as compared with pre-GFD, and with no expected deterioration in glycemic control during puberty[9]. Furthermore, the beneficial impact of GFD on BMI has been recently demonstrated, since underweight celiac patients gained weight and overweight or obese patients lost weight 2.8 years after starting on a GFD[10]. A similar effect of GFD upon lipid metabolism therefore should be considered in celiac patients, along with correction of the alterations associated with exposure to gluten in the diet. In fact, diagnosis of CD and its treatment with a GFD has resulted in improvement in the lipoprotein profile, including an increase in HDL and a decrease in the LDL/HDL ratio[11]. However, contrary results have been also published[12], although these may be explicable since following a GFD may require a restriction in carbohydrates intake, often leading to increased fat intake[13]. Depending on the quality of fat this may result in either an increase or decrease of total cholesterol and triglycerides.

Although our case represents a single observation, and larger studies are needed, it does lead us to reflect upon the importance of CD screening. IgA anti-tissue transglutaminase antibodies are considered to be the most useful markers for CD screening, but their diagnostic value in adult patients is limited[14]. In the case of children it should be noted that the anti-transglutaminase antibody titers correlate with the degree of the duodenal histological lesion, and are very low in children in whom only lymphocytic enteritis is observed. On the other hand, low antibody titres are the most common finding in the adult forms. Thus, it is estimated that their sensitivity which exceeds 90% in the case of childhood CD[15] is reduced to a mere 15%-30% in screening for CD in adults[16], for whom a positivity threshold of 2 U/mL is recommended[17]. This makes it even more necessary to obtain duodenal biopsies and conduct immunohistochemical studies to establish the diagnosis[18]. This is confirmed by the presence of a risk HLA haplotype and particularly by a good clinical and biochemical response to GFD. A strategy based on the genetic evaluation of suspect cases, followed by obtaining duodenal biopsies in individuals confirmed to have risk HLA haplotypes, makes it possible to diagnose three times as many affected cases as when the evaluation is limited to serological testing only[15]. Different experts in CD agree that because of the difficulty of establishing a diagnosis through other tests, the response to strict GFD for at least 6 mo represents the most definitive diagnostic criterion, particularly when there is an improvement or normalization of the previously altered laboratory test parameters without any associated concomitant medication[19]. Experts do not recommend the repetition of duodenal biopsies following gluten reintroduction in the case of adults with sufficient diagnostic criteria and a good response to the prescribed treatment[14].

Our patient was overweight, with an increased waist circumference, mixed hyperlipidemia, altered basal blood glucose and elevated transaminase concentrations. The traditional image of celiac patients, characterized by thin and diarrheic individuals, contrasts with the true situation, since up to 30% of all patients are overweight and 50% suffer constipation[4], as in our case.

The way in which the malabsorption alterations or immune disorders associated to CD can bring about these metabolic changes is not clear. However, the association between transaminase elevation and CD is well known, and is seen in 60% of all patients with classical clinical manifestations of the disease, as well as in 40% of atypical presentations[4]. In fact, 10% of all blood transaminase studies result in the diagnosis of CD[14]. In general, as in obesity, hypertransaminasemia in CD is due to non-alcoholic steatohepatitis and reverts following the introduction of GFD. If no reversion is observed, then the presence of primary biliary cirrhosis, sclerotizing cholangitis or autoimmune hepatitis must be excluded, as these are autoimmune conditions that are also associated to CD.

The changes in lipid profile observed in our patient following the introduction of GFD could also be explained by the dietary modifications imposed by GFD itself, a generally healthier and without precooked foods, rather than by the particular absence of gluten. However, the dietary questionnaire revealed no significant changes in diet in this sense. Likewise, the discrete changes in body weight and waist circumference do not, in isolation, seem to explain these important reductions in lipid levels, which in clinical practice are only achieved with drugs. Our patient did not actually reduce her carbohydrate intake, as is normally the case in celiac patients, where increased fat intake is usually observed when gluten-containing flour and cereals are replaced with others lacking gluten. This factor can therefore be taken to have no crucial impact upon correction of the altered basal blood glucose levels and hypertriglyceridemia. Thus, the effects of the absence of gluten upon lipid metabolism remain as the only plausible explanation and, although it must be remembered that ours is an isolated case, the findings do

invite us to investigate the metabolic changes induced by gluten in coeliac patients.

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CASE REPORT

Littoral cell angiomas of the spleen associated with solid pseudopapillary tumor of the pancreas

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Abstract

Littoral cell angiomas (LCA) of the spleen are vascular tumors of unknown etiology arising from the littoral cells of the splenic red pulp sinuses. Usually a benign and incidental finding, LCA have been repeatedly reported in association with a variety of visceral malignancies and hold the potential for dissemination *per se*. We encountered a case of a 30 year old female who was diagnosed with solid pseudopapillary tumor of the head and distal pancreas by fine needle aspiration cytology. A distal pancreatectomy with splenectomy was performed in addition to a pylorus-preserving Whipple's procedure and cholecystectomy. Histopathological examination confirmed solid pseudopapillary tumor of the pancreas and showed multiple well-circumscribed anastomosing vascular channels in the spleen. The diagnosis of LCA of the spleen was confirmed by immunohistochemistry that revealed co-expression of endothelial cell marker, CD31 and CD34, along with histiocytic marker, CD68 by the vascular lining cells. LCA has been previously reported in association with colorectal and pancreatic adenocarcinoma, malignant lymphoma, myelodysplasia and autoimmune disorders. We report the first case of LCA associated with solid pseudopapillary tumor of the pancreas.

Key words: Littoral cells; Spleen; Vascular tumors; Red pulp

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INTRODUCTION

Littoral cell angiomas (LCA) are rare vascular tumors of the spleen of uncertain biological behavior[1]. First described by Falk *et al* in 1991[2], the majority of LCA are asymptomatic incidental findings with no age or sex predilection[3,4]. Splenomegaly is a common feature of all the LCA and a few of them show symptoms of hypersple-

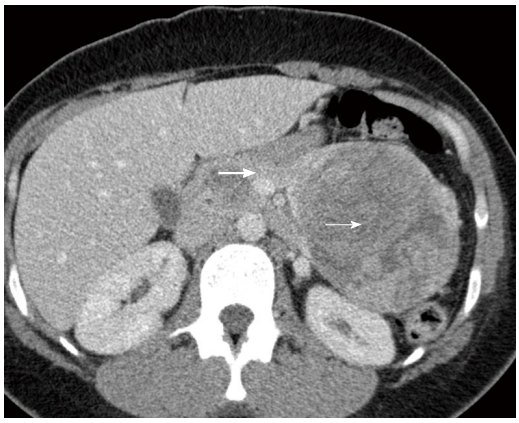

Figure 1 Computed tomography scan of the abdomen showing a large tumor in the pancreatic tail (fine arrow) and a small tumor in the pancreatic head (thick arrow).

nism[5-8]. The unique feature of almost all LCA is its immunohistochemical reactivity to CD31 (endothelial marker) and CD68 (histiocytic marker); the latter suggesting the origin of the tumor as the splenic sinus lining littoral cells. LCA have been associated with a variety of visceral malignancies, including colorectal and pancreatic adenocarcinoma, malignant lymphoma[9] and myelodysplasia[10]. To the best of our knowledge, about 35 cases of LCA have been reported to date in the English literature.

CASE REPORT

A 30 year old female with a history of sickle cell disease, SC trait presented with a 2 d history of gradual onset of back and bilateral lower extremity pain with fever and chills. The patient was diagnosed with solid pseudopapillary neoplasm of the pancreas by endoscopic ultrasonography-guided fine needle aspiration cytology. The diagnosis was confirmed by immunohistochemistry that showed a positive reactivity to CD56, synaptophysin, CD10 and alpha-1 antitrypsin. A CT-scan imaging of the abdomen identified an 11 cm tumor in the distal pancreas and a 2 cm tumor of the head of the pancreas with a bridge of preserved pancreatic tissue between the two tumors (Figure 1). A preoperative angiogram showed the dorsal pancreatic artery supplying the distal tumor and the patient underwent a distal pancreatectomy and splenectomy along with a Whipple's procedure to prevent the overt diabetes. Gross examination of the pancreas showed a yellow-tan, lobulated, well-circumscribed mass located on the anterior aspect of the pancreatic tail (Figure 2) measuring 13 cm × 10 cm × 7.5 cm and a hemorrhagic, focally cystic red-brown tumor measuring 2 cm × 1.5 cm × 1 cm in the supero-anterior aspect of the pancreatic head. Histopathological examination of the tumor in the head and the distal pancreas revealed morphological changes of solid pseudopapillary tumor. Gross examination of the spleen showed a 113 g yellowish-brown nodular organ measuring 10 cm × 6.5 cm × 3 cm. Two dark-brown, well-circumscribed nodules were identified; one measuring 1.1 × 0.7 × 0.4 cm near to the hilum and other measuring 2.5 × 0.7 × 0.3 cm just underneath the capsule. Histopathological examination showed multiple, anastomosing vascular lesions that vaguely resembled splenic sinusoids lined by tall endothelial cells (Figure 3). The vascular lesions were well delimited from the surrounding splenic parenchyma. Immunohistochemistry revealed the co-expression of CD31 (Figure 4A), CD68 (Figure 4B) and CD34 (Figure 5) by the vascular lining cells, confirming the lesion as LCA of the spleen.

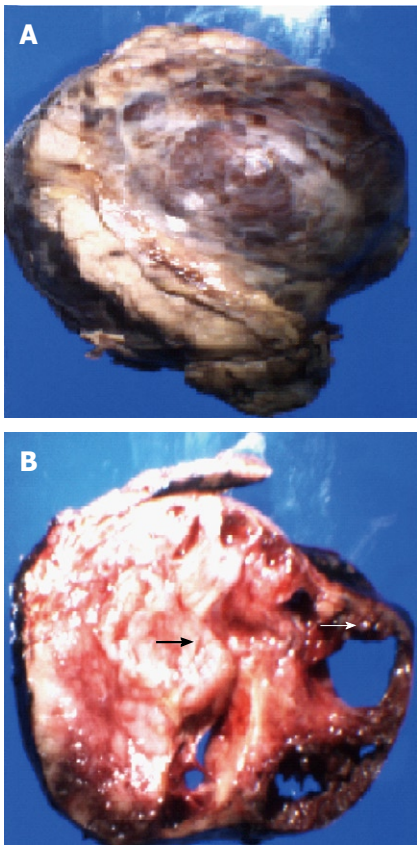

Figure 2 Gross photograph of the pancreatic tail tumor (A) and the corresponding cut surface (B) showing solid (thick arrow) and cystic areas (fine arrow)

DISCUSSION

Since the identification of LCA by Falk *et al* in 1991[2], these vascular tumors have been periodically reported[5-8] in the literature. Two forms of LCA have been described; the more commonly encountered diffuse multiple nodular form as in our case and the rare solitary form[11].

The differential diagnosis of splenic neoplasm with a radiological imaging similar to LCA is extensive and includes hemangiomatosis, lymphangiomatosis, hamartoma, hemangiopericytoma, hemangioendothelioma, angiosarcoma[1], lymphoma, metastasis and sarcoidosis.

Clinically, LCA can present as an abdominal mass, mostly due to splenomegaly, with symptoms of hypersplenism with ensuing anemia and/or thrombocytopenia, pulmonary hypertension and pyrexia of unknown origin[5-8],

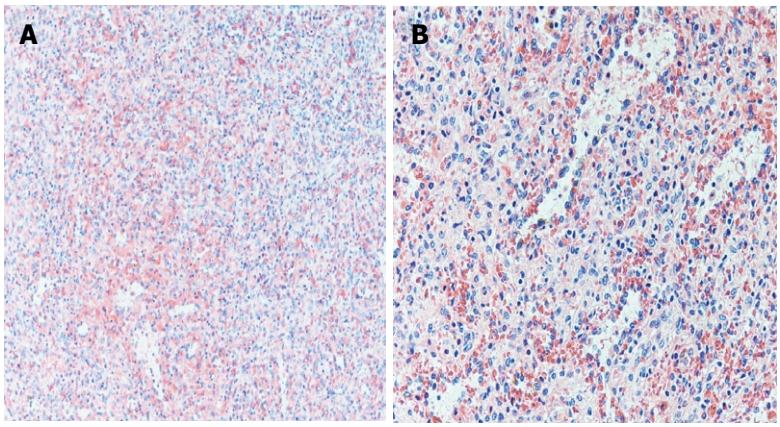

Figure 3 Histopathology of splenic nodule showing proliferation of spindle cells with anastomosing vascular channels and congestion of large vessels suggestive of LCA (Hematoxylin and Eosin stain). A: ×40; B: ×100.

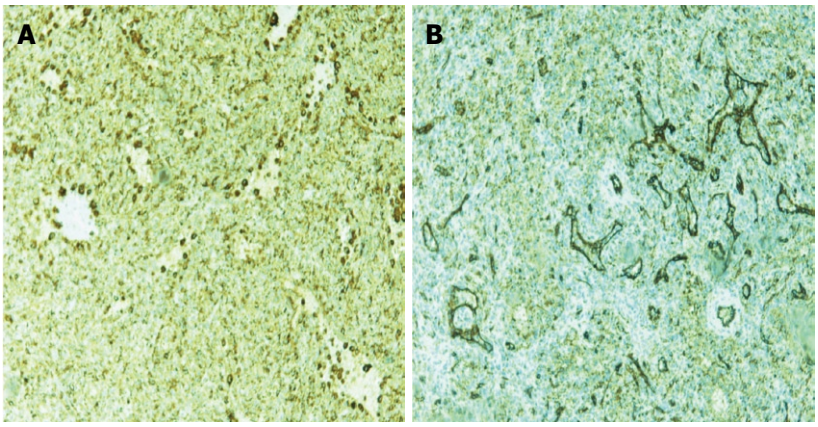

Figure 4 Immunohistochemistry of splenic nodule showing vascular lining cells reactive to CD31 (A) and CD68 (B), ×100.

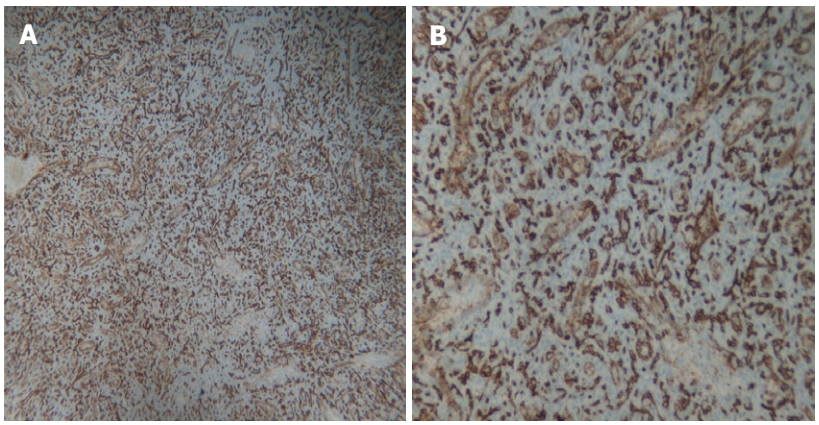

Figure 5 Immunohistochemistry of splenic nodule showing vascular lining cells reactive to CD34. A: ×40; B: ×100.

or can be an incidental finding. However, in our case the patient did not have a splenomegaly associated with solid pseudopapillary tumor of the pancreas. More dramatically, LCA has been reported to present as splenic rupture and hemoperitoneum[12,13].

Radiological studies by CT scan, MRI, sonography or nuclear medicine studies, although not conclusive[14], can contribute to diagnosing LCA. A CT-scan imaging shows LCA as hypoattenuating nodules of varying size. Delayed phase imaging on CT-scan reveals the nodules to be isodense to surrounding splenic parenchyma due to delayed filling of the nodules. MRI of the spleen shows hypodense lesions on T1 and T2 weighted scan due to the hemosiderin content of the tumor[1]. However, no hypodense nodules in the spleen were evident on CT-scan imaging in our case. Sonography is rarely helpful as findings vary greatly from isoechoic to hypo- and hyperechoic lesions[15]. Tc-99m labeled RBC scintigraphy can differentiate splenic lesions from splenic hemangiomas[16].

The pathogenesis of LCA remains unclear but, given its association with autoimmune disorders such as Crohn's disease and inborn metabolic diseases such as Gaucher's disease, immune system dysfunction has been postulated as a possible pathogenic mechanism[17,18]. Supporting this hypothesis, other reports have suggested that chronic infection and systemic immunosuppression may contribute to the development of LCA[12,19]. Interestingly, once thought of as a benign and incidental lesion, one third of the reported cases are associated with malignancies of visceral organs including adenocarcinoma of colorectum (most

common), kidney, liver[20], lung[21], pancreas, hepatocellular carcinoma and malignant lymphoma[9]. It has also been associated with myelodysplasia[10] and aplastic anemia[22]. Interestingly, this is the first report of a LCA of the spleen associated with a solid pseudopapillary tumor of the pancreas. The strong association of LCA with various malignancies necessitates splenectomy in most of the cases. The splenectomy in our case, however, was a part of the distal pancreatic tumor excision.

Two subtypes of LCA, angiosarcoma and hemangioendothelioma, have been reported to have malignant potential. In two rare cases[23,24], distant metastasis with neoplastic cells consistent with the morphology of LCA have been identified after splenectomy. There was no evidence of any distant metastasis of the LCA in our case.

The definite diagnosis of LCA is made at pathology after splenectomy which remains the gold-standard of the treatment[13]. Grossly, the spleen shows nodules with blood/blood products of variable color, usually dark-red to brown/black depending upon the chronicity of blood in these lesions[16]. Histopathology reveals proliferation of anastomosing vascular channels lined by tall endothelial cells with papillary fronds extending into the vascular channels. Some exfoliated cells may be seen in the vascular spaces and atypical cells and mitoses are rare. LCA shares morphological and immunohistochemical features with hemangiomas at other locations such as immunoreactivity for vascular endothelial marker CD31 and factor Ⅷ. Even although they are usually negative for markers highlighting the red pulp sinusoidal epithelium such as CD8 and CD34, the LCA in our case expressed the endothelial marker, CD34 (Figure 5). The expression of endothelial marker CD31 and histiocytic marker CD68 by the vascular cells is unique and diagnostic of LCA[1], as in our case.

In conclusion, LCA are primary vascular neoplasms of the spleen and are usually an incidental finding. Even though the vast majority of these are benign, malignant association and potential have been documented prompting close evaluation and surveillance in patients with LCA for development of other malignancies. We report the first case of an incidental LCA of the spleen associated with a solid pseudopapillary tumor of the pancreas.

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Parimal Chowdhury's work on smoking related pancreatic disorders

Parimal Chowdhury

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Figure 1 Parimal Chowdhury (left), PhD, Professor, University of Arkansas for Medical Sciences Department of Physiology and Biophysics, 4301 W Markham Street, Little Rock, AR 72205, United States.

Abstract

Cigarette smoking is a known risk factor for the development of numerous diseases. The role of nicotine in the induction of pancreatic inflammation and pancreatic cancer as a result of cigarette smoking has been recognized and reported in the literature. The mechanism by which nicotine induces such pathologies is as yet unknown. An understanding of the proliferative potential of nicotine in primary and tumor cells of the pancreas will allow us to develop measures that will ultimately lead to intervention, prevention and treatment of these diseases. Studies show that nicotine can increase the cell numbers of certain cancer cell lines, suggesting that exposure to nicotine can lead to the disruption of the dynamic balance between cell death and proliferation, which is required for normal functioning of cells. We hypothesize that nicotine induces oxidative stress in pancreatic acinar cells and thus contributes to this disruption. We have used the AR-42J cell line in our study because of its stability as an immortal tumor cell line and its known physiological similarity to primary acinar cells. Our studies show that mitogen activated protein kinase signaling is induced by nicotine in AR42J cells, causing an increase in lipid peroxidation and a subsequent decrease in cell function. Our data suggest that exposure to nicotine induces oxidative stress, leading to cell injury and compromised function, thus implicating cigarette smoking as a plausible mechanism.

Key words: Nicotine; AR42J cell; Effect on mechanisms; Lipid peroxidation; MAPK signaling; Cell function

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INTRODUCTION AND EDUCATIONAL EXPERIENCE

Dr. Parimal Chowdhury PhD. (Figure 1) is currently working as Professor of Physiology & Biophysics and Associate Professor of Pharmacology & Toxicology at the University of Arkansas for Medical Sciences (UAMS), Little Rock, Arkansas. He also has a joint appointment as Adjunct Professor in the Department of Applied Science at the University of Arkansas at Little Rock (UALR). Before joining the UAMS in 1980, Dr. Chowdhury worked as Assistant Professor at the University of Medicine & Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey. He graduated with B.Sc. and M.Sc. from Dacca University. Later, Dr Chowdhury moved to Canada and earned his Ph.D. degree in Immunochemistry/Physiology in 1970 from the prestigious McGill University, Montreal, Canada.

Dr. Chowdhury's research is focused on the study of how nicotine, a major component in cigarette smoking, induces patho-physiological changes in the exocrine pancreas. Applying physiological, biochemical, ultra structural and molecular techniques, his group focuses on the implications of associated studies that explain the development of pancreatitis in rats caused by nicotine which lead to pancreatic carcinogenesis. Dr. Chowdhury is also concentrating on developing an animal model of simulated weightlessness following induced microgravity, and to study the physiological responses of various tissues to hind limb suspension with the ultimate aim of developing a countermeasure for space travel-related sickness. Dr. Chowdhury' s research projects have been supported in part by funds from the NIH, the Arkansas Space Grant Consortium, and other agencies.

Dr. Chowdhury has authored/co-authored over 117 peer-reviewed research publications including book chapters, and editorials in high-impact journals such as *AJP, Ann Clin Lab Sci, Gastroenterology, J Pancreatol, JBC, J Biomed Optics, J Cell Physiol, J Clin Immunoassays, J Lab Clin Med, JPET, J Surg Res, Pancreas, Exp Biol and Med, Science*, *World J Gastroenterol* as well as many others. He has had over 260 scientific research abstracts published, from presentations at various local, national and international conferences. He serves on the Editorial Board of the *World Journal Gastroenterology and the World Journal of Gastrointestinal Pathophysiology*, and is a reviewer for numerous peer-reviewed journals.

Dr. Chowdhury has traveled extensively worldwide, and has delivered lectures as visiting scientist/professor. He is an active member of various national/international societies and has served on many committees. He served as President of the International Society for the Prevention of Tobacco Induced Diseases (ISPTID), an international scientific society (2006-2008), and also as President of the Association of Scientists of Indian Origin in America (ASIOA), a scientific society in the USA (2006-2008). He is also a member of the Central Arkansas Chapter of the Society of Sigma XI and represents Arkansas at the national meeting each year. He has been a recipient of many competitive awards and honors throughout his career. Dr. Chowdhury has supervised and/ordirected many students for their thesis and/or dissertation projects.

ACADEMIC STRATEGY AND GOALS

Cigarette smoking is a risk factor for many diseases[1-5], including alcoholic and chronic pancreatitis, and has been suggested as the single most important factor for the induction of pancreatic diseases[6,7]. Nicotine, a major component of the particulate fraction of cigarette smoke, is ingested via smoking of cigarettes or the use of other forms of tobacco. It is a known addictive agent, a drug which can be abused, and a procarcinogen. In animal studies, nitrosamines and N-nitroso-nornicotine, chemicals found in tobacco smoke, appear to be carcinogenic in the pancreas. Studies show that nicotine can increase the cell numbers of certain cancer cell lines[8-10]. It is our belief that pathophysiological changes in the pancreas in smokers, leading to the development of pancreatitis, are caused primarily by nicotine from the cigarette smoking.

Studies from our laboratory have shown that rats exposed to nicotine via oral and aerosol routes develop changes in pancreatic function and histology that are consistent with the onset of pancreatitis. Further studies are designed to critically evaluate the effects of nicotine in the development of pancreatitis in rats.

We hypothesize that rats exposed to nicotine will develop pancreatitis and that the pathological injury in the pancreas induced by nicotine is caused by alterations in cellular, subcellular and/or genetic mechanisms.

Over the years Dr. Chowdhury's research group has developed an animal model of pancreatic injury by nicotine in rats, who have been exposed to it through either via inhalation or ingestion. Subsequent molecular analysis has led to a hypothesis of identification of predisposed pancreatitis development by nicotine, leading to pancreatic oncogenesis. Dr. Chowdhury's group has approached the nicotine-induced effect further through mitogen-activated signaling pathways involving transcription factors. The current research strategy addresses the oxidative effect by nicotine at cellular level, leading to injury and compromised pancreatic function. We have used the AR42J cell line in our study because of its stability as an immortal tumor cell line and its known similarity in physiological characteristics to primary acinar cells[11].

ACADEMIC ACHIEVEMENTS

The following few selected contributions highlight Dr. Chowdhury's activities in the field of nicotine induced pancreatic injury and dysfunction.

Tissue distribution of [^{3}H]-nicotine in rats

We have determined that the pancreas is a target organ for nicotine accumulation. Distribution of [^{3}H]-nicotine in rats, after a bolus injection of nicotine, or a continuous infusion of nicotine, has revealed that the accumulation of nicotine was highest in the kidneys, regardless of the route of administration. Retention of nicotine by constant

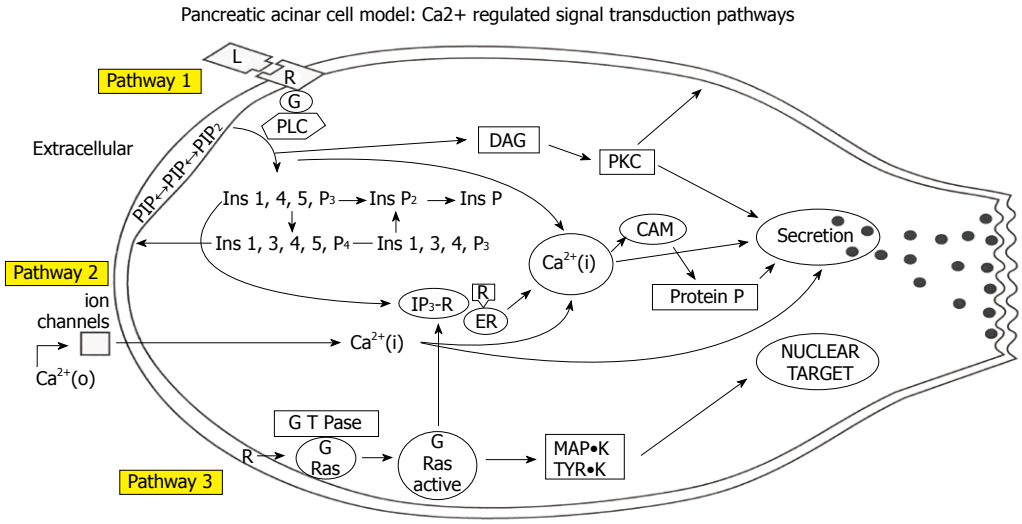

Figure 2 Pancreatic acinar cell model. A pancreatic acinar cell model showing the multiple signal transduction pathways is proposed that will replicate signal transduction pathways of a normal acinar cell described previously by other investigators[14-16]. Known agonists and antagonists acting directly on acinar cell surface receptors (pathway 1), ion channels (pathway 2), and intracellular receptors (pathway 3) are utilized to delineate the mechanism.

infusion was significantly higher in most organs, including the pancreas (6.4% ± 0.5% [^{3}H]-nicotine/g tissue), compared to retention resulting from the bolus injection (5.2% ± 0.4% [^{3}H]-nicotine/g tissue). These results indicate that the length of time of exposure to nicotine can be associated with the amount of nicotine that is stored in the organs of rat.[12]

Induction of pancreatic acinar pathology via the inhalation of nicotine

Rats exposed to aerosolized nicotine *via* inhalation induced onset, progression, and sequential development of lesions in the pancreas. Experimental groups were exposed to saline or nicotine for 15, 30, 45, and 60 min, twice a day, for 21 d. Results showed that pathological pancreatic lesions remained confined to the exocrine pancreas. The effects on pancreatic histology and plasma levels of nicotine were shown to be related to the time of exposure.[13]

Mechanism of action of nicotine on the exocrine pancreas

The mechanism by which nicotine induces pancreatic pathology is unknown. However, studies from our laboratory strongly indicate the involvement of the exocrine pancreas rather than the endocrine pancreas as a potential target for injury. A pancreatic acinar cell model X that will replicate signal transduction pathways of a normal acinar cell described previously by other investigators[14-16] is proposed in Figure 2. Known agonists and antagonists acting directly on acinar cell surface receptors, ion channels, and intracellular receptors will be utilized to delineate the mechanism. A schematic diagram showing the multiple signal transduction pathways is also shown in Figure 2 (pathways 1, 2, 3). Using this model, we plan to examine each of these distinct transduction pathways in a systematic manner.

CONCLUSION

The successful completion of these studies will provide an animal model for the study of this disease and should provide important information that could aid health care providers in establishing methods for the control, diagnosis, and treatment of pancreatitis.

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January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

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Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

March 03-05, 2011
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March 07-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

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British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

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MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 06-07, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 07-09, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 07-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV
SPIGC, II ESYS, Napoli, Italy

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
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September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
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19th United European Gastroenterology Week, Stockholm, Sweden

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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