Original Communication

Polymorphism of alpha-1-antitrypsin and association of S and Z alleles with duodenal ulcers

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Background: Duodenal ulcers are mucosal erosions that penetrate into the muscularis propria of the duodenum. They are a result of an imbalance between aggressive and defensive factors. Various environmental factors like *Helicobacter pylori* infection, addictions to smoking and alcohol etc. and genetic factors have been reported to be associated with duodenal ulceration. Alpha-1-antitrypsin was studied for its role as a genetic marker and specific allelic association to protein functioning and alteration. Serum samples from 185 normal subjects and 210 duodenal ulcer cases were typed for the phenotypes following PAGE (polyacrylamide gel electrophoresis) and immunofixation using specific commercial antisera with appropriate staining protocols. In general, 'M' allele of alpha-1-antitrypsin was found to be predominant in healthy normal subjects, with the gene frequencies being 0.679 (M), 0.299 (Z) and 0.0214 (S). Whereas in duodenal ulcer cases, Z and S alleles were found to be predominant with a significant association of MS, ZZ and MZ phenotypes ($\chi^{2:}$ 49.98) and the gene frequencies being 0. 113 (M), 0. 347 (Z) and 0.506 (S). Predominance of Z and S alleles indicates that these alleles may encode for reduced synthesis of alpha-1-antitrypsin, hence decreased neutralization of proteases like trypsin and chymotrypsin inhibited by alpha-1-antitrypsin, thereby resulting in ulcers. The study highlights the association of Z and S alleles of the potent protease inhibitor alpha-1-antitrypsin and also suggests its role as a genetic marker in ulcerogenesis.

Key Words: Protease inhibitor, tissue degradation, glycoprotein, genetic marker

Introduction

Duodenal ulcers are lesions on the mucosa that extend into the muscularis propria of the duodenum. They occur in the first part of the duodenum. An imbalance between aggressive (acid, pepsin) and defensive (mucosal defenses) factors is the primary event for the disruption of mucosal integrity leading to ulcerogenesis.

Duodenal ulcers have been reported to be a net result of environmental and genetic factors with the mode of inheritance being multifactorial.[1] Earlier studies reported an association of blood group O,[2] ABH non - secretor status,[3] HLA-B5 phenotype,[4] Gc 1-1 phenotype[5] with duodenal ulceration. Hyperpepsinogenemia (PG) was reported to be a sub-clinical risk factor in duodenal ulceration.[5-7] The role of Cathepsin E and alpha-1-antitrypsin as defensive factors in duodenal ulceration[5,8] was also reported.

Alpha-1-antitrypsin, a major protease inhibitor that protects the mucosal tissue from degradation is found to be localized on chromosome 14 q 31. Trypsin and Chymotrypsin are the two proteases confirmed by earlier studies as etiological factors in duodenal ulceration that are known to be inhibited by alpha-1-antitrypsin. Recent studies have shown the association of S and Z alleles of alpha-1-antitrypsin with deficiency of the enzyme.[9] Thus, the study envisages into the polymorphic variation of alpha-1-antitrypsin and its role as a genetic marker in ulcerogenesis.

Materials and Methods

With the approval of the local ethics committee, the

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study envisaged the analysis of blood samples from 210 endoscopically confirmed duodenal ulcer patients, and 185 normal subjects (without prior medication) that were collected from the Gastroentererology unit of Osmania General Hospital, Hyderabad, by visiting the hospital twice a week (Mondays and Thursdays) during the period of study (1998-2001). No patient was excluded from the study. Apart from blood samples, epidemiological data regarding the socio economic stata, familial history and food habits including addiction to smoking and alcohol were collected from each of the individuals of both the disease and control groups. The blood samples were allowed to coagulate and immediately centrifuged at 2000rpm for 5 minutes. The serum samples obtained after centrifugation were analysed for the phenotypes of alpha-1-antitrypsin, carried out on 7% polyacrylamide gels following polyacrylamide gel electrophoresis (PAGE).[10] The glass plates with polymerized separating gel were fixed to a vertical slab gel electrophoretic unit. The upper and lower tanks containing the tank buffer were connected to the anodic and cathodic ends of the DC power supply. 40 ml of serum with 20ml of bromophenol blue indicator were loaded in the sample wells. Electrophoresis was conducted at 20 mA constant current at 4°C for 4-5 hours. The gel was then subjected to immunoblot with alpha-1-antitrypsin antisera (Sigma grade) and was incubated overnight at 4°C that was later washed and stained[11] with simultaneous destaining until distinct bands of alpha-1-antitrypsin were visible on a clear background.

The data was computed and analysed for gene frequencies and Woolf's test of association was carried out to identify any specific electromorphic association with duodenal ulcers.

Results

The frequency distribution of alpha-1-antitrypsin electromorphs in control and disease groups presented in Table 1 indicates a general predominance of M allele in normal subjects and Z and S alleles in disease condition. In normal subjects, 44.0% were of MM type, followed by MS (16.0%), MZ (12.0%) and ZZ (8.6%) phenotypes. While in duodenal ulcer cases, 32.0% were of MS, 23.3% were ZZ and 22.0% were of MZ phenotypes.

Table 1: Frequency distribution of alpha-1-antitrypsin electromorphs in control and duodenal ulcer groups

Phenotypes	Control		Duodenal ulcer	
	n	%	n	%
ZZ	19	8.6	49	23.3
MZ	23	12.0	47	22.0
MM	82	44.0	12	6.0
MS	29	16.0	67	32.0
FS	16	9.0	9	4.0
SZ	1	0.5	13	6.2
SS	-	-	13	6.2
FM	16	9.0	-	-
FF	2	1.0	-	-
Total	185	-	210	-

Test of association of alpha-1-antitrypsin phenotypes in duodenal ulcer cases is presented in Table 2. Relative risks estimates (RR) of various phenotypes were calculated of which MM phenotype when compared with MZ, MS and FM revealed significance with the relative risks being 0.09 (χ^2: 49.98*) and ZZ phenotype when compared with SZ and MZ phenotypes had relative risks of 1.2 (χ^2: 0.28) with no significant association. Similarly, homozygotes when compared with heterozygotes revealed a significant predisposition of heterozygotes to duodenal ulcer, with the relative risk being 0.46 (χ^2: 13.84*) (*: P<0.01).

The allelic frequencies of M, S and Z phenotypes of alpha-1-antitrypsin are presented in Table 3. The gene frequencies were calculated and were observed to be

Table 2: Relative risk estimates (RR) of alpha-1-antitrypsin phenotypes in control and duodenal ulcer groups

Phenotype	Control	Duodenal ulcer	RR	χ^2
ZZ	16	49	-	-
ZZ *Vs* MS, MZ	24	60	1.2	0.28
MM	82	12	-	-
MM *Vs* MZ, MS, FM	68	114	0.09	49.98*
SS	-	13	-	-
SS *Vs* SZ, FS, MS	46	89	-	-
FF	2	-	-	-
FF *Vs* FS, FM	32	9	-	-
ZZ, MM, SS, FF Vs MZ, MS, FS, SZ, FM	100 85	74 136	- 0.46	- 13.84*

*P<0.01

Table 3: Allelic frequencies of alpha-1-antitrypsin phenotypes In control and duodenal ulcer groups

Phenotype	Genotype	Observed		Expected		χ^2		Allelic frequencies	
		Control	DU	Control	DU			Control	DU
M	MM, MS, MZ	134	126	164	42.9	22.2	p	0.679	0.113
Z	ZZ, MZ, SZ	43	109	94.68	65.6	28.7	q	0.299	0.347
S	SS	-	13	0.09	53	30.2	r	0.0214	0.506

*DU: Duodenal Ulcer
p,q,r: haplotypes M,Z and S.

0.679(M), 0.299(Z) and 0.0214(S) in control subjects and 0.113(M), 0.347(Z) and 0.506(S) in duodenal ulcer cases.

Discussion

The predominance of S and Z alleles in the disease condition compared to the control group, observed in the present study suggests that these alleles may encode for reduced synthesis of alpha-1-antitrypsin, which can be strengthened by earlier studies.[9] It can also be thought on the lines that, any abnormalities of the liver and its metabolism may also lead to reduced synthesis of this glycoprotein since the liver is the site of alpha-1-antitrypsin synthesis. As glycoproteins work to protect the surface of the gastric mucosa against the effects of acid and toxic metabolites, the alteration may thereby result in failure to render mucosal cytoprotection. These conditions may make the gastric mucosa more susceptible to autodigestion by tissue degrading proteases like trypsin and chymotrypsin due to the inability of alpha-1-antitrypsin to form molar complexes with them. Hence, decreased protection conferred to the gastric mucosa from the proteolytic enzymes highlights the role of alpha-1-antitrypsin as a genetic marker in ulcerogenesis. This also indicates an imbalance between aggressive and defensive mechanisms. Thus, predominance of S and Z alleles of alpha-1-antitrypsin may help as a preclinical marker to aid in early treatment. Although, the study implicates to explore the role of other genetic and environmental factors influencing the process of ulcerogenesis.

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