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Low Serum Alpha 1 Antitrypsin in Duodenal Ulcer - A Family Study

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Introduction

Genetic association of a disease is often recognized due to its occurrence in families. Duodenal ulcer like many common diseases has a variable age of onset, expression and familial aggregation¹. Three lines of evidence support a genetic role: family studies, twin studies and blood group studies. Family aggregation is commonly associated with early onset (<30 years) and blood group O with late onset of symptoms². The disease has a genetic component that predisposes and an environmental factor that helps in its manifestation¹.

Alpha-1 antitrypsin (alpha I AT) deficiency is an inherited metabolic disorder associated with not only a severe reduction of alpha-AT in blood but also amino acid substitution of alpha-I AT due to gene variations³. Subjects with alpha I AT deficiency⁴ particularly its Z allele are more prone to develop duodenal ulcers⁵. This association may be a contributory factor towards the aggravation of the disease and this perception becomes even stronger when other risk factors for duodenal ulcer like helicobacter pylori are absent among the family members. Z mutation is the most common genetic defect in alpha AT deficiency and its screening is commonly done by Isoelectric focusing (IEF). However, for accurate identification genetic screening has been recommended which includes detection of inherited traits by measurements of enzyme activities in blood, the presence of a specific gene or a specific mutation⁴. We, therefore have applied both the methods and have found that polymerase chain reaction (PCR) based method is an effective way to confirm the phenotypes⁶.

Case Report

In Pakistan, duodenal ulcer is infrequent in females especially those who are not taking non-steroidal anti-inflammatory drugs (NSAID)⁷. The proband, a female developed ulcer symptoms at the age of 50 years. Upper gastrointestinal (GI) endoscopy at that time showed streaks of esophagitis with no abnormality in stomach and duodenum. After a year she was re-endoscoped as her symptoms had worsened. This time apart from esophagitis, a large anterior wall duodenal ulcer with contact bleeding was seen, she was treated with Ranitidine (300 mg) at night for a month. Repeat endoscopy after 4 weeks showed a healing duodenal ulcer (smaller than before). The drug was continued for 8 weeks and then reduced to half the dose at bed time for another 8 weeks. Patient was asymptomatic for almost 9 months, when her symptoms recurred along with epigastric tenderness. Ranitidine was given for another 8 weeks, after which she again became asymptomatic. She had multiple relapses since then, each treated with H2 receptor antagonists. On last relapse she also complained of vomiting and inability to retain food. Upper GI endoscopy showed linear streaks of severe esophagitis with bile reflux, elongated stomach with billious gastric residue and pyloric stenosis. At this stage patient refused surgery and started self-medication. Tests for helicobacter pylori were negative. It was therefore considered appropriate to screen for various genetic markers i.e., alpha 1 AT, serum pepsinogen and blood group. Written consent was obtained from all the patient and her family members and an approval was granted by the ethical committee of the Jinnah postgraduate medical centre, Karachi to study genetic markers. Serum sample drawn from patient was subjected to radial immunodiffusion (RID)⁶ and (IEF)⁸ for the estimation of serum alpha-I AT levels and determination of phenotypes

respectively. The concentration of alpha-I AT by RID was less than 50% of the normal level (any value less than 2 gm/l was considered below normal). Phenotyping revealed a deficiency variant that was inferred to be ZZ phenotype as no prominent protein bands were visible in the area designated for alpha-I AT indicating the presence of a partial to total alpha-I AT deficiency. Further confirmation of phenotyping was done by deoxyribonucleic acid (DNA) analysis techniques. DNA was extracted⁹ and a combination of PCR and restriction enzyme digestion¹⁰ was then applied to confirm the identity of deficient variant. Her blood group was AB and serum pepsinogen concentration using the method of Edwards et al¹¹ was above the normal expected range i.e., 179 units/ml (normal range: 60-160 units/ml).

The family history of the proband showed that her parents were first cousins, among the immediate family, one of her brother had a duodenal ulcer. All the other siblings and offsprings screened for serum alpha I AT by the same methods were healthy adults with no signs and symptoms of duodenal ulcer.

None of the sibling and offsprings had helicobacter pylori as judged by the CLO test¹².

Serum samples from three siblings, two daughters and one son were analyzed. The results are presented in the accompanying Table and Figure 1.

Table. Serum alpha 1 antitrypsin by RID and IEF in the family of a duodenal ulcer patient.

	Age (years)	Sex	IEF Phenotype	alpha-AT g/l
Proband	56	F	ZZ	1.46*
Sibling 1	48	M	MM	2.60
Sibling 2	43	M	MM	2.60
Sibling 3	50	M	?	1.46*
Son	22	M	ZZ	1.20*
Daughter ₁	24	F	MM	2.00
Daughter ₂	26	F	?	1.46*

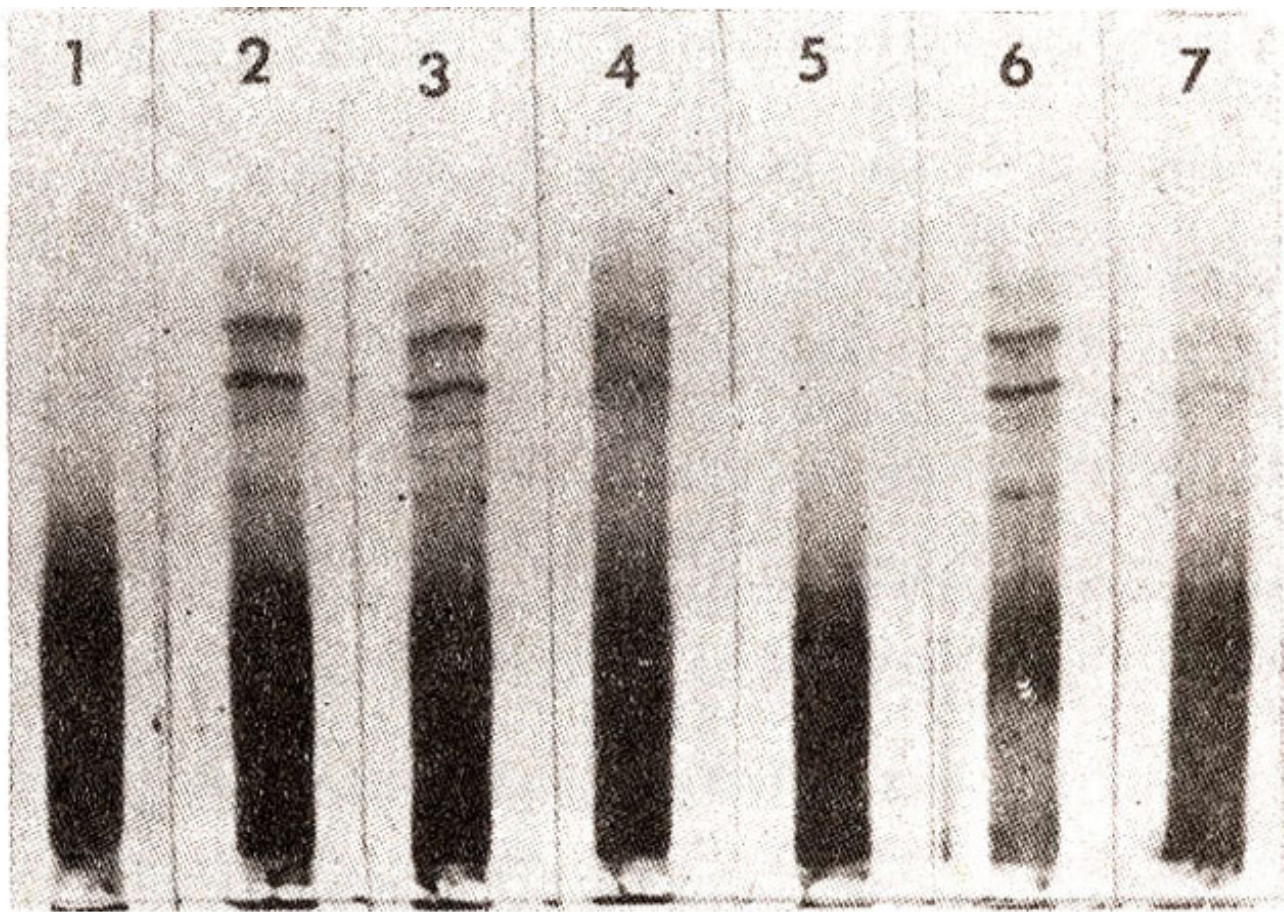


Figure 1. Phenotypes of alpha 1 AT in the family of a duodenal ulcer patient. IEF showing alpha 1 AT phenotypes at pH 4-5 Proband (channel 1), siblings (2-4), son (5), daughters (6,7). Anode at top.

One of the sibling had a low level on the partigen plate and IEF showed a pattern that looked like a heterozygous variant that was not clearly resolved into MM or MZ. Other two siblings showed normal variants of M by IEF.

One of the daughters also had a normal variant and normal concentration of alpha-I-AT whereas the son and the other daughter and low levels of alpha I AT. This daughter also exhibited a pattern on IEF that appeared indistinct. The son demonstrated a pattern on LEF which was very similar to that of the proband and clearly exhibited a pattern of ZZ type. To confirm the IEF results, particularly in one of the siblings and daughter with low alpha 1 AT, molecular techniques were applied which not only provides early identification but also distinguishes heterozygous and homozygous alpha-i antitrypsin deficient¹³. The results of the typing for the Z mutation more clearly established the presence of Z mutation in the proband and her son. The relatively unresolved phenotyping carried out by IEF of one sibling and a daughter who had low alpha-I AT levels with indistinct patterns showed that the daughter and the sibling both had MM phenotypes. as judged by PCR and subsequent restriction digestion. Thus normal persons and those homozygous for the Z mutation were distinguished unambiguously by applying PCR based method. All the subjects with normal MM phenotype believed to have the normal sequence as their amplified product by PCR and subsequent digestion with restriction enzyme Taq I produced a DNA fragment of 64 base pair, that is only observed in the absence of a mutation. While the proband and her son's DNA treated in the same way manifested Z mutation by showing a final product at the 86 base pair position as analyzed on agarose gel (Figure 2).

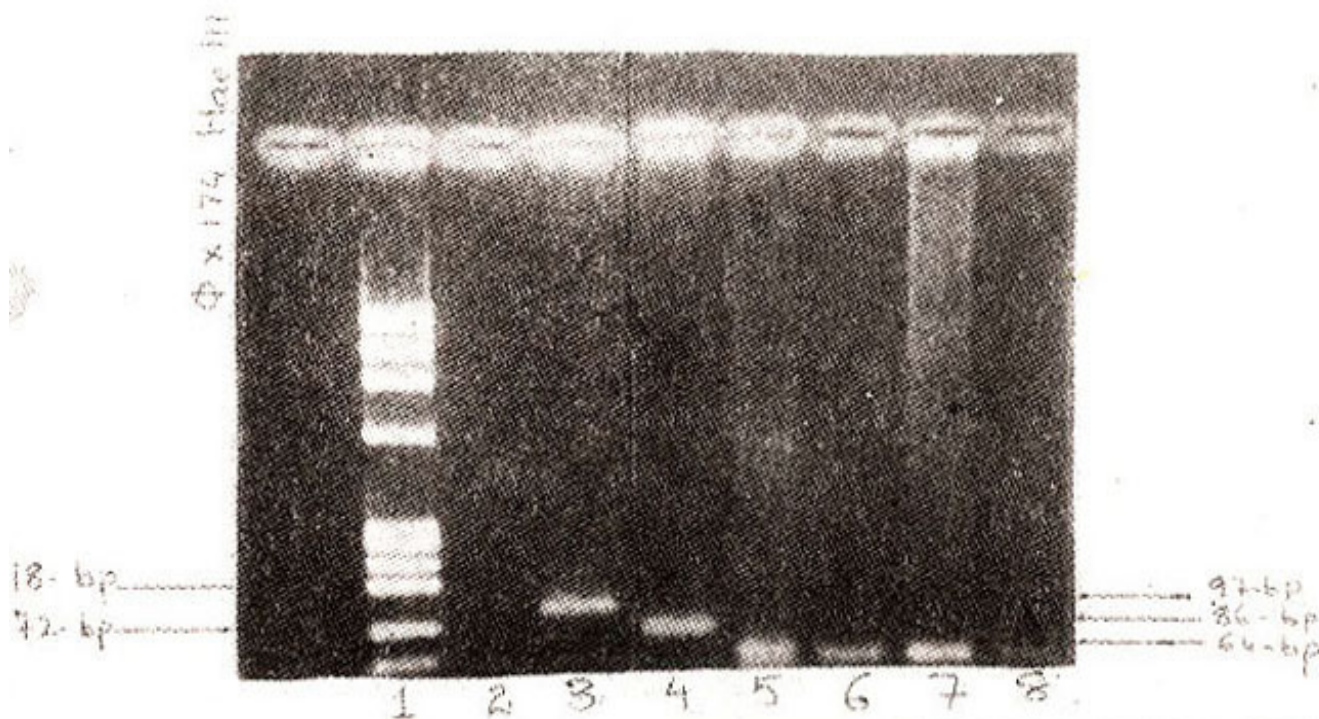


Figure 2. Agarose gel electrophoresis after digestion. Ethidium bromide stained agarose gel after electrophoresis of Taq 1 digested and undigested products. Ladder (channel:1), Bland z (2), Uncleaved (97 bp) PCR product Z(3), cleaved fragment with mutated (86 bp) sequence (4) and cleaved fragment with normal (64 bp) sequence (5,6,7,8)

In our experience IEF should be used as the screening method and the DNA based techniques as the verification of cases where serum concentration is low and IEF does not clearly discriminate the Phenotypes.

Discussion

Duodenal ulcer is a common gastrointestinal problem in this part of the world and a search for the causative and aggravating factors is in progress. Relatively few evidences support a positive correlation between alpha1 AT deficiency and duodenal ulcer and these are mostly of preliminary nature^{4,5}. We were able to identify an ulcer patient, with low serum alpha-I AT with a deficient variant on LEF, which was further confirmed by molecular techniques. This patient also had a raised serum pepsinogen. These observations suggested the etiology to be of genetic origin. Such an association with genetic markers demonstrates the importance of genetic factors and also serves in the detection of genetically predisposed persons¹. Recent advances in molecular genetics have improved our understanding of the disease and these scientific advances have important implications in the diagnosis, treatment and screening of patients with genetic diseases and their families¹⁴. Considering the patient's follow up which was almost over six years and in spite of treatment with H2 receptor antagonists, she had relapses of ulcer. The genetic origins of ulcer disease could have been one of the factors responsible for a poor response to therapy leading to frequent relapses in the absence of H. pylon. It is possible that due to alpha- I AT deficiency, activity of proteolytic enzymes remained unchecked and resulted in frequent recurrences¹⁵. In the present study more than 50% of the offsprings and one sibling also manifested low levels of alpha-I AT. All these were healthy adults with no signs and symptoms of duodenal ulcer. This situation is very much similar to an earlier reported study¹⁶ where by using serum pepsinogen as a genetic marker in duodenal ulcer; it was suggested that the family members of patients

with raised serum pepsinogen should be screened to identify those carrying the trait, so that they could be protected from such factors that may induce and/or aggravates the disease process. The same hypothesis may hold true in the present study where it could be suggested that family members of duodenal ulcer patient carrying that trait are at an increased risk for duodenal ulcer.

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