

Analysis of GSTM1 and CYP1A1 genes in tropical chronic pancreatitis: a pilot study

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ABSTRACT

Background: The etiopathogenesis of tropical chronic pancreatitis (TCP) remains unknown. Oxidative stress and exposure to environmental toxins (xenobiotics) have been proposed as risk modifiers.

Objective: The objective was to study the prevalence of selected abnormalities in the xenobiotic metabolizing genes in subjects with TCP.

Methods: Twenty-one patients attending the Pancreas Clinic at our center were studied and the results of gene analysis were compared with a control group of 400 healthy volunteers. The outcome studied was the prevalence of deletions and mutations in the xenobiotic-metabolizing genes GSTM1 and CYP1A1 and the DNA repair gene XRCC1 in subjects with TCP.

Results: Our results show that 9.5% of subjects with TCP had deletions of GSTM1 and 33% had polymorphisms of CYP1A1. In the control group (n=400) the prevalence of these polymorphisms were 28% and 16% respectively. Compared with controls, polymorphisms of CYP1A1 were commoner in subjects with TCP, though this was not statistically significant (p=0.064). The prevalence of GSTM1 polymorphisms was higher in the control group, but this too was not statistically significant (p=0.077).

Conclusions: These results of this small pilot study do not imply that a genetic susceptibility to environmental disruptors could be an important factor in the pathogenesis of TCP. Larger studies are needed to assess the links between these genes and TCP, especially considering that both are associated with a higher risk of pancreatic cancer. [IJEM 2008;12(5):3-6]

Key Words: Endocrine disruption, pancreatic diabetes, oxidative stress, xenobiotic, chronic pancreatitis, pancreatic cancer

INTRODUCTION

The etiopathogenesis of Tropical chronic pancreatitis (TCP) has been a mystery(1,2). Malnutrition and cassava intake had been the preferred hypotheses earlier, but these hypotheses have now been strongly questioned(3,4). In recent times, genetic factors have led to a paradigm shift in the understanding of the illness(5). TCP is increasingly being recognized as a heterogeneous disease with multiple risk factors(3,5). These risk factors could either be an extrinsic toxin, or an intrinsic abnormality increasing the subjects' vulnerability to extrinsic factors.

Xenobiotics (environmental toxins like cigarette smoke

and occupational chemicals) have been proposed as extrinsic factors involved in pancreatitis(6). Xenobiotic compounds include solvents, fuels, phenols, polyaromatic hydrocarbons, herbicides, and halogenated alkanes. However their mere presence in the atmosphere is not sufficient, and increased susceptibility to these xenobiotics, it has been postulated, could be conferred by micronutrient deficiency and oxidant stress(7). Studies on the anti-oxidant status of subjects with TCP have shown abnormal antioxidant status in these subjects(7,8) Micronutrient antioxidants react with glutathione that is present in tissues to accelerate disposal of reactive oxygen species as well as xenobiotic metabolites that may be derived via the cytochromes P450 pathway(7). Pancreatitis can be caused by the heightening of oxidative-detoxification reactions induced by cytochrome p450-1 activity in the liver or pancreas. Theophylline clearance, which is a marker of cytochrome p450-1 activity in vivo, has already been shown to be increased in subjects with TCP(9).

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Therefore, we postulated that genetic defects that increase the damage caused by environmental disruptors could play a role in the genesis of TCP. We studied the role of 2 genes important in the susceptibility and recovery from xenobiotic-induced oxidative stress: the two i.e. *GSTM1* and *CYP1A1* are important in oxidative-detoxification of environmental toxins and have already been implicated in the pathogenesis of pancreatitis(6). This study was carried out in the state of Kerala in India, which is the region with one of the highest prevalence rates of subjects with TCP in the world(10). In addition, Kerala is also a state with high susceptibility to xenobiotic exposure as it has a very high load of pesticide toxicity(11).

METHODS

We studied 21 patients with tropical chronic pancreatitis (TCP), based on the following criteria: (1) recurrent pain (2) large intraductal calculi, particularly in the head region; (3) ultrasonological and ERCP evidence of pancreatic calcification; (4) absence of any other etiological factors like alcoholism etc; (5) diabetes mellitus (may or may not be present). After obtaining informed consent, EDTA blood samples were collected from 21 patients. Genomic DNA was isolated from peripheral blood leucocytes following standard protocols. The methodology used for genotype analysis of the *CYP1A1* and *GSTM1* was Polymerase Chain Reaction (PCR) with specific primers, using well-described protocols used by the collaborating center in earlier studies (Table

1)(12-14). We also carried out genotype analysis of these genes in 400 healthy control subjects derived from the healthy volunteers.

The Institutional Ethics Committee had cleared the study. The two groups (cases and controls) were compared using Fischer's Exact test to compare frequencies. Two-tailed *p* values less than 0.05 were considered statistically significant.

RESULTS

The mean age of the subjects was 43.4 +/- 12 years. Seven among the 21 subjects were females and 14 were males. Eleven subjects were of a lower socioeconomic status, while 10 subjects were of a middle-income socioeconomic status. Six out of the 21 subjects gave a history of cassava intake. Four among the 21 were smokers. Two subjects with TCP had developed pancreatic cancer (Table 2).

The results of the genotype analyses are shown in table 1. Deletions of the *GSTM1* were found in two (9.5%) subjects. Seven (33%) subjects had polymorphisms in the *CYP1A1* gene, of which one was of a homozygous type, the rest being heterozygous. In a series of 400 blood samples obtained from healthy volunteers who have had no incidence of cancer or any other known systemic disease, we genotyped *CYP1A1* and *GSTM1* genes. The *GSTM1* gene was deleted in 28% (n=112/400) of the normal population and actively present in 72%. For the *CYP 1A1* gene 16% (n=64) of the 400 normal population were polymorphic, of which 2% were

Table 1: Methodology used for PCR for *GSTM1* and *CYP1A1*

GSTM1 Analysis

PCR was carried out in a total volume of 50 microliter containing 0.5-1.0 microgram of genomic DNA, 120 ng of each of the primers (forward & reverse) for *GSTM1* and beta-globin (control gene), PCR buffer (1X) [50mM KCl, 10mM Tris-HCl (pH 9.0), 1.5mM MgCl₂ and 0.1% Triton X-100], 200 micromolar of each dNTP and 1.25 units of Taq DNA polymerase. Negative controls consisted of a similar reaction mixture with the template replaced with sterile water. Initial denaturation at 94 deg C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 64°C for 60 sec and extension at 72 deg C for 60 sec. A final extension was carried out at 72°C for 5 min.

The presence of 232 bp (*GSTM1*) and 265bp (beta-globin) bands implies the presence of *GSTM1* while the presence of only 265bp band implies *GSTM1* deletion.

Primer sequence:

<i>GSTM1</i> :	A: 5'-GAA	CTC	CCT	GAA	AAG	CTA	AAG	C-3'
	B: 5'-GTT	GGG	CTC	AAA	TAT	ACG	GTG	G-3'
Beta-globin:	C: 5'-CAA	CTT	CAT	CCA	CGT	TCA	CC-3'	
	D: 5'-GAA	GAG	CCA	AGG	ACA	GGT	AC-3'	

CYP1A1 m1 & m2

10 microliters of the purified PCR product was digested with 20 units of *MspI* (*CYP1A1 m1*) and *NcoI* (*CYP1A1 m2*) restriction enzymes at 37 degrees C for 1 hour. The RFLP products were then electrophoresed on an agarose gel and visualized using ethidium bromide staining. 100bp DNA molecular weight marker was used to assess the size of the PCR-RFLP products.

Evaluation of RFLP:

<i>MspI</i>	200 & 140bp => homozygous variant
(<i>CYP1A1m1</i>)	340, 200 & 140 bp => heterozygous variant.
	340 bp => wild type
<i>NcoI</i>	232 bp => wild type
(<i>CYP1A1m2</i>)	232 & 263 bp => heterozygous variant.
	263 bp => homozygous variant

Table 2: Analysis of the GSTM1 and CYP1A1 genes

Case No.	Remarks	GST M1	CYP 1A1
1	56 year male	PRESENT	WILD
2	15 year old girl with diabetes	PRESENT	WILD
3	30 year old male	DELETED	HETEROZYGOUS POLYMORPHIC
4	65 year old lady with diabetes	PRESENT	WILD
5	30 year old male takes Cassava	PRESENT	WILD
6	42 year old male with diabetes takes Cassava smoker	PRESENT	WILD
7	52 year old lady with diabetes, takes Cassava	PRESENT	HETEROZYGOUS POLYMORPHIC
8	53 year old male with Diabetes takes Cassava	PRESENT	WILD
9	23 year old lady severe pain, required stenting	PRESENT	WILD
10	23 year old lady obstructive jaundice	PRESENT	WILD
11	67 year old male	PRESENT	WILD
12	36 year old male pancreatico-jejunostomy for recurrent pain	PRESENT	HETEROZYGOUS POLYMORPHIC
13	31 year old male	PRESENT	HETEROZYGOUS POLYMORPHIC
14	51 year old male with diabetes smoker	PRESENT	WILD
15	51 year old male with pancreatic cancer	PRESENT	WILD
16	42 year old lady with diabetes	PRESENT	WILD
17	56 year old lady with Diabetes	PRESENT	WILD
18	51 year old male with pancreatic cancer smoker	PRESENT	WILD
19	39 year old male with diabetes takes Cassava	PRESENT	HETEROZYGOUS POLYMORPHIC
20	19 year old male	PRESENT	HETEROZYGOUS POLYMORPHIC
21	47 year old male smoker, takes Cassava	PRESENT	HOMOZYGOUS POLYMORPHIC

homozygous polymorphic and 14% were heterozygous polymorphic. As compared with the control population, the prevalence of CYP1A1 polymorphisms was higher in the subjects with TCP (NS; $p = 0.064$; CI=95%), but this did not reach statistical significance. The difference in the prevalence of GSTM1 too was not statistically significant when subjects with TCP were compared with healthy controls. ($p = 0.077$). Overall, 7 out of the 21 subjects (33%) had an abnormality in either the CYP1A1, or GSTM1 or both.

DISCUSSION

The results of this small study suggest that environment-disrupting genes are present in about one third of subjects with TCP. However, there was no statistically significant difference when compared with a control population. Hence our results largely negate, but do not

completely rule out the effects of these functionally relevant polymorphisms in low penetrance genes. It is possible that these genes might cause additive or even synergistic effects on an individual who already has other risk factors for TCP. This fits in well with the current model of TCP as a heterogeneous, multifactorial disorder. Our study is limited

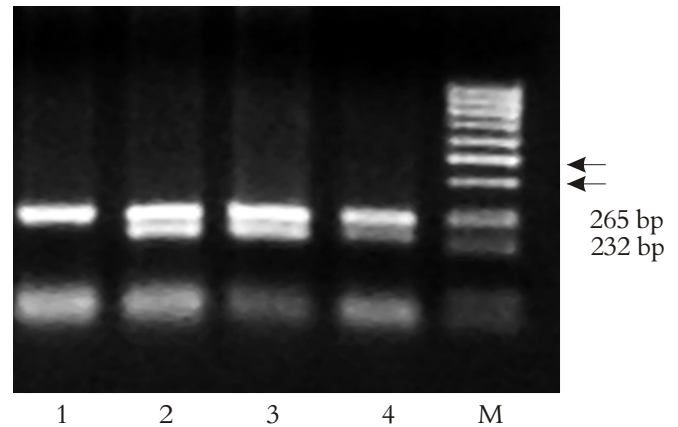


Figure 1. Ethidium bromide stained agarose gel showing PCR products corresponding to the alleles of the GSTM1 gene. "M" is the DNA Size marker. Lanes 2, 3 and 4 show presence of the GSTM1 gene, and Lane 1 shows deletion of the gene.

by the small sample size, which prevents us from making statistically significant conclusions. The small sample size also meant that correlations between the genotype and phenotype would not be possible.

The glutathione S-transferases (GSTs) are a family of

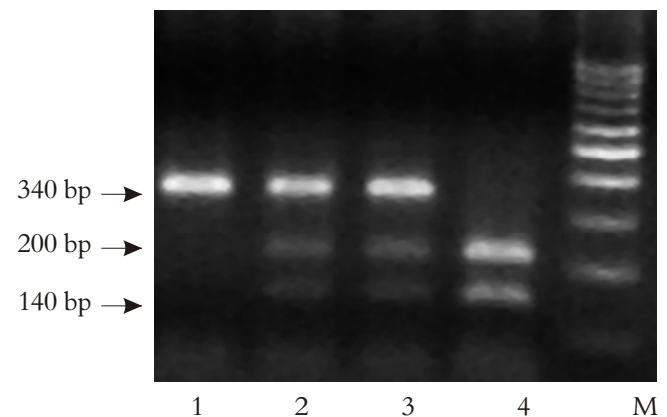


Figure 2. Ethidium bromide stained agarose gel showing PCR products corresponding to the alleles of the CYP1A1 genes. Lane 1 shows the wild type, lanes 2 and 3 show a heterozygous polymorphism, while lane 4 shows a homozygous polymorphism

enzymes that help in detoxifying a wide range of chemicals and thus protecting the body from oxidative stress, including carcinogens. In some GST genes there are polymorphisms, which alter the activity of these enzymes. GSTM1 genes exhibit deletion polymorphisms. Homozygous deletions of those genes, called GSTM1 null genotypes, result in a lack of enzyme activity. A decrease in GST enzyme activity could

result in inefficient detoxification of various carcinogens, which could lead to genetic damage and increased cancer risk(15). GSTs have already been linked to pancreatic cancer(16). In our study, the *GSTM1* deletions were present in only about 10% of the subjects, lower than the value of 28% in controls; this suggests that *GSTM1* does not appear to be an important gene in this setting of TCP. *GSTM1* has been linked to chronic alcoholic pancreatitis(6). In a recent study, it was shown that GSTM1 is not involved in the pathogenesis of hereditary pancreatitis(17). The role of *GSTM1* null mutations in chronic pancreatitis is controversial, and indeed, whether they protect or predispose to injury itself is a subject of debate(18,19).

One of the most interesting outcomes of this study was the increased incidence (though not statistically significant) of polymorphisms in the CYP 1A1 gene. Polycyclic aromatic hydrocarbons (PAHs), a class of chemicals that includes potent carcinogens, have been postulated to have a role in the genesis of TCP(3). Automobile exhaust, industrial emissions and smoke from burning wood, charcoal and tobacco contain high levels of PAHs. The major metabolic pathway for ingested or inhaled PAHs to water-soluble derivatives is oxidative activation by CYP1A1 followed by detoxification by phase II enzymes. If these are not removed from the body by this system, PAHs and their metabolites cause genetic and tissue damage(20). Most environmental carcinogens undergo initial metabolism by CYPs (Phase I enzymes) into either inactive metabolites or into chemically reactive electrophilic metabolites which can bind to DNA and trigger a carcinogenic response; these reactive metabolites may be converted by both Phase I enzymes or by Phase II enzymes (e.g., GST) into inert, and biologically inactive products(21). Very recent studies of CYP1A2 genes have shown that they could be an important factor in the pathogenesis of pancreatic cancer(22). In the only Indian study looking into this aspect, theophylline kinetics, a marker for the potentially toxic CYP-450I pathway of drug metabolism, was studied in 11 controls and 11 patients with TCP: the results showed that theophylline clearance was faster among subjects with TCP, suggesting that the toxic pathways were activated(9).

Our results justify the need for a larger study, preferably a case control study with a large sample size studying multiple environment-disrupting genes, to assess the genotype-phenotype correlations. Such genotype analyses might detect high-risk profiles, and may even facilitate future diagnostic and intervention strategies for the disease.

Disclosure: The results of this study have been presented in the Amrita Journal of Medicine, which is the in-house (private circulation) journal of the Amrita Institute of Medical Sciences.

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