Original Article

Distribution profile of paraoxonase phenotypes among the Gujaratis

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BACKGROUND: Paraoxonase (PON1) can hydrolyze organophosphate pesticides (OP) and has a key role in the susceptibility of human in OP toxicity. The human-enzyme shows polymorphism and variations in the distribution profile of its phenotypes among different ethnic groups have been observed.
AIMS: To see the distribution pattern of total PON1 activity in 45 healthy attendants of poisoning cases; 121 healthy unrelated farm-labours and 59 normal subjects of trauma.
MATERIALS AND METHODS: The PON1 activities from serum/plasma samples of these healthy normal individuals were estimated with/without addition of 1M NaCl in order to determine salt-stimulated and basal activity. The PON 1 phenotypes were determined on the basis of percent activation of enzyme activity.
RESULTS: Tri-modal distribution of basal PON1 activity was observed among all these individuals. 52.0% of the individuals belonged to Phenotype A, 46.6% to phenotype AB while 1.4% to Phenotype B with gene frequency of allele-A and allele-B being 0.753 and 0.247 respectively in excellent agreement with Hardy-Weinberg equilibrium.
CONCLUSION: Maximum number of individuals belonged to phenotype-A (low PON1 activity) showing potential vulnerability towards Op-poisoning.

Key words: Paraoxonase, phenotypes, Gujaratis

Introduction

Human paraoxonase (EC 3.1.1.2) is an HDL-associated serum enzyme synthesized in liver whose preliminary physiological role is to protect low density lipoprotein (LDL) from oxidative modification.[1] Its family contains at least three members: PON1, PON2 and PON3 of which PON1 is studied well.[2,3] A number of lines of evidence suggest that PON1 present in the blood of mammals can protect against exposure to OP compounds (OPs) by hydrolyzing them to relatively harmless excretable products.[4,5] It is capable of hydrolyzing multiple substrates including organophosphate pesticides, nerve agents, oxidized lipids and a number of drugs or pro-drugs.[6] Recently, it has been shown to have a key role in the susceptibility of humans in OP toxicity due to its genetic polymorphism.[7]

It is having two polymorphisms in the coding region and five polymorphisms in the promoter region. The Q isoenzymes (phenotype-A), with glutamine at position 192, has low activity towards paraoxon whereas the R isoenzymes (phenotype-B), which has arginine at position 192, possess high activity towards paraoxon.[8] On the basis of enzymatic tests, humans could be divided into three serum (PON 1) phenotypes: phenotype-A (low activity), phenotype-AB (intermediate activity) and phenotype-B (high activity) without a clear demarcation between intermediate and high metabolizers.[9] Variations in the distribution profile of these phenotypes among different ethnic groups have been observed. It has been shown to have unimodal distribution in Negroid and Mongoloids, bi-modal in European population[10] while West Germans and White Americans showed tri-modal distribution.[11,12] Tri-modal distribution of total PON1 activity is also observed in the Indian population from Bombay and the surrounding area and among North-West Indian Punjabis.[13,14] But no such reports are available on the Gujarati-Indian population. Present study was undertaken to study the distribution of total PON1 activity in this population.

Materials and Methods

Subjects

The study group consisted of 45 healthy attendants (40 M, 5 F) of poisoning cases referred to our centre (Group-I), 121 healthy unrelated male farm laborers

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from Rupal Village of Gujarat (Group-II) and 59 cases of trauma (26 M, 33 F) from civil hospital, Ahmedabad (Group-III). All the individuals included in the study were from Gujarat and were healthy normal individuals with their age ranging from 14-72 years. Prior written consent was taken from the individuals before collection of the blood samples. Individuals with the history of IHD, hypertension, diabetes mellitus, hyper-lipidaemia or malignancy were excluded from the study.

3-4 ml Blood samples were collected by venepuncture in plain/heparinized vacutainer tubes. The samples were centrifuged at 5°C at 2000 rpm for plasma/serum separation which were then stored frozen at -20°C for PON 1 analysis within a week.

The PON 1 activities were measured by modified method of Eckerson using paraoxon as the substrate.[15] The PON1 activities from plasma/serum samples were estimated with/without addition of 1M NaCl in order to determine salt-stimulated and basal activity. The rate of hydrolysis of Paraoxon was assessed by following the liberation of p-nitrophenol measured by the increase of absorbance at 412 nm on a spectrophotometer (Cary100, Varian) at 25°C. The basal assay mixture included 1 mM paraoxon and 1 mM $CaCl_2$ in 0.05M glycine-buffer (pH 10.5) and that of salt stimulated PON1 assay included 1 M NaCl in addition to this mixture. One unit was defined as the amount of PON1 producing 1 nmol of p-nitrophenol per minute per milliliter serum. The percent stimulation of PON1 was calculated as:

$$\frac{\text{Paraoxon activity with 1M NaCl - Basal PON1 activity} \times 100}{\text{Basal PON1 activity}}$$

The PON 1 phenotypes were determined on the basis of percent activation of enzyme activity by 1 M NaCl: Phenotype-A (< 60% activation), Phenotype- AB (60-200% activation) and Phenotype-B (> 200% activation).[15]

Paraoxon and glycine-HCl were obtained from Sigma (St Louis, MO) and the other chemicals used in the assay were of analytical grade.

The statistical method used was the Chi-square test. Statistical significance was established at $P < 0.05$.

Results and Discussion

Our results showed tri-modal distribution of basal PON1 activities in the groups of normal Gujarati-individuals from three different sources [Figure 1]. It also showed tri-modal pattern when data of these groups were pooled [Figure 2]. When total individuals were divided by assigning phenotypes on the basis of percent activation of the enzyme by 1M NaCl; 52.0% of them showed < 60% activation (Phenotype A), 46.6% had 60-200% (Phenotype AB) while 1.4% showed > 200% activation (Phenotype B) with gene frequency of allele-A and allele-B being 0.753 and 0.247 respectively in excellent agreement with Hardy-Weinberg equilibrium [Table 1]. Mean activities of plasma/serum PON1 in these phenotypic groups were found to be 121.05 ± 3.19; 272.89 ± 5.37 and 469.71± 21.23 U/L (Mean ± SEM) in the Phenotype-A, Phenotype-AB and phenotype-B groups respectively. Maximum number of the individuals (52.0%) studied belonged to A-phenotype (low activity group) on the basis of percent

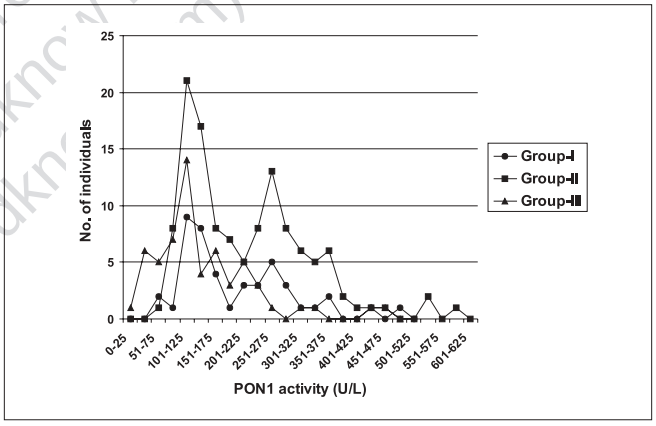

Figure 1: Distribution of basal paraoxonase activity among three different groups of healthy Gujarati individuals

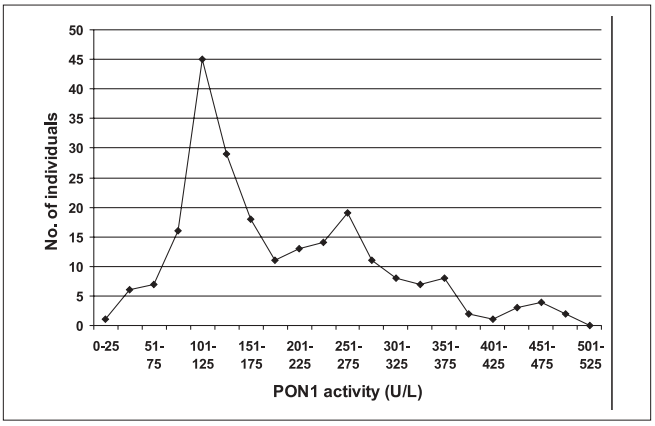

Figure 2: Distribution plasma - basal PON1 activity among healthy Gujarati individuals (all the three groups pooled, N=225)

Table 1: Distribution of paraoxonase phenotypes and allelic frequency among healthy Gujarati individuals based on salt-activated (1M NaCl) enzyme activity

Observed number of individuals with each phenotype[@](O)	Observed allele frequencies	Expected genotype frequency	Expected number of individuals (E)	Observed -expected individuals (O-E)
Phenotype-A = 116 (52.0%)	Allele-A (p) = 0.753	P^2 =0.57	127.11	-11.11
Phenotype-AB = 104 (46.6%)	Allele-B (q) = 0.247	2pq =0.37	82.51	21.49
Phenotype-BB = 03 (1.4%)		q^2 =0.063	14.05	-11.05
Total 223 individuals		$P^2 + 2pq + q^2 = 1$		χ^2 = -0.67, NS

NS = Non-significant, [@]Based on % activation by 1M NaCl; Phenotype A: < 60% activation; Phenotype AB: 60-200% activation and Phenotype BB: > 200% activation by 1M NaCl, The figures in parenthesis indicate the percentage of individuals

activation by 1M NaCl. Sanghera *et al.* have reported tri-modal distribution among Asian-Indians in America; 47% of them belonging to genotype-AA and 13% to the genotype-BB with gene frequency of 0.67 and 0.33 for gene-A and -B respectively.[16] In north-western Indians the distribution was found to be tri-modal with frequency of allele-A and allele-B as 0.845 and 0.155 respectively.[14]

Based on their study on eighteen agricultural male workers who were exposed to a variety of OPs in Turkey, Akgur *et al.* proposed that human subjects with phenotype-A are probably more susceptible to OP poisoning than those with -AB or B-phenotypes.[17] Growing interest in PON1 arises from the hypothesis that individuals with low serum activity of this enzyme would be expected to have a diminished ability to metabolize oxon forms and therefore might be more susceptible to the toxicity of OP.[18] Turks, Palestinians, Iranians, Indians and Sri Lankans like Europeans, have a larger group of low PON1 activity.[13] In our study, majority of the individuals (52.0%) studied belonged to A-phenotype (low activity) and it is higher than the lower activity subpopulation reported for the English (49%),[19] Canadians (44%),[20] Koreans (19%), Japanese and the Indonesians (10%), Nigerians (6%), Zimbabweans and Zambians (0%).[11] Recently, efforts are underway to identify genes and polymorphism that play an important role in 'environmental susceptibility' and PON1 polymorphism has been cited as a prime example of such a genetic polymorphism.[21] Since the poisoning due to OP-poisoning being most frequent one in the countries like India and Srilanka and majority of their population belonging to the low PON1 activity group which is expected to increase the burden on the health services; we believe this study to have greater implications.

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