Original Article

Pseudo-cholinesterase polymorphism in Mazandaran province (North of Iran)

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Abstract

Background: Pseudocholinesterase (PChE) polymorphism has been a subject of several pharmacogenetic studies worldwide. The patients with atypical homozygous genotype do not only have reduced serum cholinesterase activities but also their elimination rate for some pharmacologically potent drugs decrease drastically. This study was designed to evaluate the PChE polymorphism in Mazandaran province (northern Iran) for the first time.

Methods: About 5 ml plasma samples were collected from 200 healthy volunteers who visited "Iran Blood Transfusion Organization" centers all across Mazandaran province for blood donation. The PChE activity in presence or absence of dibucain was measured and based on obtained dibucain number (DN) volunteers were categorized to normal homozygous (Eu,u), atypical heterozygous (Eu,a), and atypical homozygous (Ea,a).

Results: The average (±SD) of the PChE activity among the blood donors was 9.14±1.93 IU (ranging from 4.1 to 16.6 IU). Only 2 persons (1%) had DN between 60 to 70 (Eu,a) and no one was categorized in 20<DN<30 (Ea,a) group. Nine volunteers (4.5%) in normal group (Eu,u) had DN values less than 72. The within- run and between run-precisions (CV) for DN measurements in our laboratory were found to be 2% and 4%, respectively.

Conclusion: According to the findings, only 1% of the sample population had atypical heterozygous phenotype taking into account the experiment precision, the frequency of Ea,u is less than 4%. These findings suggest that the atypical allele frequency in northern Iran's population is probably less than the other regions of Iran and some other countries.

Keywords: Pseudocholinesterase, Polymorphism, Succinylcholine, Mivacurium

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There are two major types of cholinesterase enzymes, which are mainly distinguished in terms of tissue distribution and enzymatic properties, including inhibition by different drugs (1). The first group, which is found in the synaptic clefts and in the red blood cells, is called truecholinesterase. The second one is dominantly distributed in plasma and other tissues including the liver and called pseudo-cholinesterase or according to its preferred substrate, butyryl-cholinesterase (also known by other names: plasma or serum cholinesterase) (2-6). The pseudocholinesterase enzyme (EC3.1.1.8) is a tetrameric glycoprotein with 574 amino acid residues and 9 carbohydrate chains attached to each unit. It is the product of a 73 KB gene on chromosome 3 with 3 introns (2, 5, 7).

Several mutant forms of PChE enzyme have been discovered that some of them cause significant reduction in the activity of this enzyme (enzyme deficiency) and clinically can lead to an increased apnea length (secondarily to adminstration of some muscle relaxants) (2). Using enzyme based kinetic assays, variants of PChE enzyme have been identified by 4 allele genes; U (general or wild), A (atypical or resistant to dibucaine), F (resistant to fluoride) and S (silent or negative) categorized in 10 phenotypes (8-11). The prevalence of atypical form differs among populations.

About 3% of European descendants are atypical heterozygous for this variant (Eu,a) (12). However, since this gene is recessive, no special conditions exist in these people except for a minor decrease in PChE enzyme activity which is not clinically important. On the other hand, in homozygous individuals (Ea,a) who have a prevalence of about 0.3%, the enzyme activity drastically decreases and these patients are at risk of prolonged apnea after the administration of succinylcholine (13-14). The percentage of PChE inhibition by dibucaine in different enzyme phenotypes (dibucaine number) is used to identify the polymorphism of this enzyme (2). Normal people (with genotypic Eu,u) show a 70-80 percent PChE inhibition by dibucaine (DN=70-80) while this percentage is 50-60% in Ea,u and 20-30% in Ea,a phenotypes (1).

The atypical phenotype was introduced in 1957 by Kalow et al. (15). The prevalence of atypical homozygous phenotype (Ea,a) in white Americans is about 1 in 2,000. In a study conducted in 1996 in Trinidad by Pinto et al. it was shown that people with African ancestry have less of PChE enzyme defect compared to Indians and other races (16). There are few reports of lower frequency of atypical alleles in China and Africans with the black race (9, 17).

While it was believed that the frequency of PChE enzyme deficiency in white Europeans is more than Asians (18), some studies have suggested a higher prevalence in Iranian population (18-19). For example in a study conducted by Hosseini et al. in 1997, the prevalence of PChE deficiency among an Iranian sample was higher than the Irish population (19).

In another study, Szeinberg et al. have suggested that the Iranian Jews have high frequency of atypical allele (7.6%) (20). In a study conducted in Kermanshah (a province at north-west of Iran), different PChE enzyme phenotypes were studied in 1548 persons and the frequency for atypical heterozygous (phenotype AU) and atypical homozygous (phenotype AA) were found to be 3.8% and 0.2%, respectively (9), which was much lower than 7.6% reported by Szeinberg.

The prevalence of PChE enzyme defeciency in Mazandaran province has not been studied so far and the information about whole Iranian population are limited and contradictory.

The aim of this study was to determine the prevalence PChE enzyme (atypical phenotype) in Mazandaran province as a major Iran subpopulation.

Methods

Plasma Samples: The plasma samples were collected from 200 healthy blood donors aged 18 to 50 who had visited 5 centers of "Iran Blood Transfusion Organization" (IBTO) in Mazandaran province (Amol, Babol, Behshahr, Ghaemshahr, and Sari), North of Iran. All donors had consented to give an extra blood sample for PChE study. They were healthy adults born and living in Mazandaran province with no drug consumption history in the last 2 weeks. More than 4 ml plasma samples were collected from 10 ml heparinized blood samples (by centrifuging 10 ml of blood at 400 g for 15 minutes) and quickly transferred to the laboratory on ice. The samples were aliqueted in 1 ml microtubes and stored in -20^{o}C and the polymorphism experiments were conducted in less than 3 months.

Determination of PChE activity and Dibucaine number (DN): The PChE activity and DN were measured using Ellman method with minor modifications (21-22). Briefly, the optical density (OD) of Dithio-bis-nitro-benozoic acid 5,5'-dithiobis (2-nitrobenzoic acid (DTNB) reaction with thiocholine, product of acethyl-thiocholine hydrolysis by PChE, for 10 minutes at 37 ^{o}C was measured in the absence or presence of dibucaine.

Chemical reagents: All the chemicals used in this experiment were purchased from Sigma-Aldrich (UK). Color-substrate buffer (CSB) was prepared freshly by dissolving 48 mg acethyl-thiocholine iodide (AcT) and 20 mg DTNB in 12 ml potassium phosphate buffer (0.2 M, pH=8) (PPB). Quinidine sulphate (QS, 300 mg/ml) in PPB was used as a stopping solution. Dibucaine (4 mg/ml) in distilled water (DW) was used for DN measurement.

Assay procedure: Three glass tubes were labelled as B (for blank), D (for dibucaine), and W (for water) and 0.6 ml of CSB solution was added to each tube. In tubes B and D, 50 µl dibucaine solution, and in tube W, 50 µl DW were added. After thoroughly mixing for 5 minutes in a 37^{o}C water bath, 100 µl of 2 times diluted plasma in distilled water was added into tubes D and W, while in tube B only 100 µl DW was added. After 10 minutes incubation in 37^{o}C, 10 ml stopping solution (QS) was added to each tube. The OD of tubes W and D were measured at 450 nm using tube B content as blanking solution by a spectrophotometer (Camspec®, UK). The obtained ODs were used to calculate PChE activity and DN for each donor's blood sample.

PChE activity calculation: The activity of plasma cholinetsrease, as the mmol of AcT hydrolysed by 1 ml of

plasma during 1 minute at 37°C, was calculated using the following formula:

PChE Activity (IU) = [δA/10] × F × E_{450}

Where: IU is PChE Activity unit (mmol/min/ml), [δA/10] absorbance changes during 10 minutes, F final dilution factor in the cell, and E is the molar absorbance coefficient of TNB at 450 nm (13.6 $mM^{-1}.cm^{-1}$).

DN calculation**:** To determine the dibucaine number (DN) of each sample, the PChE acitivty in the presence (D) and absence of dibucaine (W) was measured and the following formula was used:

DN (%)= (W –D / W)× 100

Blood donors, based on their DNs were categorized into normal homozygous (Eu,u) (70≤DN≤90), atypical heterozygous (Eu,a) (60≤DN≤70), and atypical homozygous (Ea,a) (20≤DN≤30). Based on each phenotype frequency in 200 samples, their percentage was calculated.

Statistical analysis: The PChE acitivty corresponding to each IBTO's center was reported as mean±SD. To compare PChE activities among the different IBTOs centers, one-way ANOVA with post-hoc test was used.

To calculate the within-run and between-run variations, PChE acitivty of 5 samples were measured either 3 times during the day or during 3 different days (2 weeks apart). Using the following equation, the mean of corresponding CVs was reported as within-run and between-run precision.

CV (%) = (SD/mean) × 100

Where: CV and SD are coefficient of variations and Standard Deviation of 3 repeats, respectively.

Results

The PChE activity mean (±SD) of the 200 blood donors in Mazandaran province was obtained to be 9.14±1.9 IU ranging from 4.1 to 16.6 IU (figure 1). There were no significant differences among the PChE activities obtained at different IBTO's centers (P>0.05). The frequency of the donors with different percentages of PChE inhibition by dibucaine (DN) showed that no one is categorized as homozygous atypical (20<DN<30), 2 persons were categorized as heterozygous atypical (60<DN<70), and 198 persons had homozygous normal phenotype (Figure 2). According to these findings, only 1% (2 donors) of the population had heterozygous atypical phenotype. One of these two donors was a 38-year man from Babol (DN=66.7) and the other was a 47-year man from Sari IBTO center. The frequency of donors phenotypes according to their DNs are depicted in figure 2. The mean of within-run and between-run variations for DN determination were 1.4% and 3.6%, respectively.

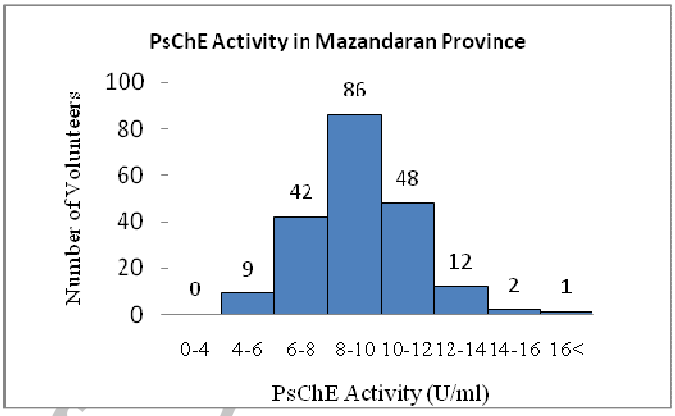

Figure 1. The frequency of pseudocholinesterase (PchE) activity among 200 volunteers in Mazandaran province (north of Iran)

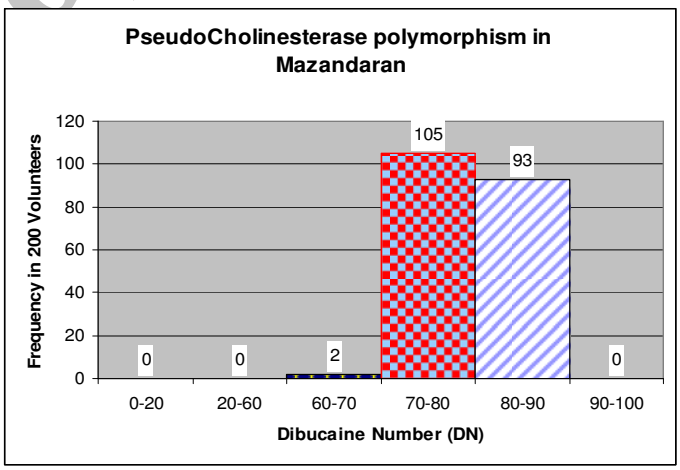

Figure 2. Dibucaine numbers (DN) of 200 volunteers in Mazandaran province (north of Iran) (The DN between 70 to 90 indicates homozygous normal and those between 60-70 are heterozygous atypical. No one categorized as homozygous atypical (DN between 20 to 30)

Discussion

The pseudo-cholinesterase activity has an important impact on pharmacokinetics of some drugs used in anesthesia and its congenital defects, especially when inherited as homozygous, can lead to fatal elongation of corresponding drugs duration of action (1, 14, 23-24). Although at least six different variants of this enzyme have

been discovered so far, but among them, the atypical variant has more prevalence and importance in pharmacogenetic studies.(1, 25) Based on the obtained results in this study, only 2 individuals from a total of 200 volunteers (1%), had heterozygous atypical phenotype. Since the PChE activity of these two volunteers remained in the normal range, the muscle relaxants elimination rate was not affected. According to the previous studies, patients with heterozygous atypical phenotype do not show siginificant reduction in PChE activity (1). Nevertheless, a reduction as big as 75% of the normal value in PChE activity does not clinically lead to significant adverse effects (23).

The biochemical method used for genotyping the volunteers in this experiment was an internationally validated method which seemed to be quite informative for clinical evaluation of patients phenotypes. However the use of PCR technique for determining the different variants, although more expensive and elaborative, is superior (1). Also the enzyme activity assay used in this study was based on endpoint colorimetric assay, while kinetic based assays using Peltier chamber with higher precision and accuracy could be more reliable.

The obtained prevalence of heterozygous atypical genotype in Mazandaran province (1%) is less than its reported frequencies in Iran and some other parts of the world. For instance, in a similar study conducted in the West of Iran, the prevalence of this genotype ($E_{a,u}$) obtained to be 3.7% (9). In another study, the prevalence of PChE atypical was reported to be higher (about 4%) among Iranians compared to an Irish sample (19). Nevertheless, it is higher than the reported frequencies for East Asians (0.73%) or black Africans (0.46%) (9) In a study conducted in Trinidad, in 1996 by Pinto et al. a lower frequency of PChE enzyme defects was observed among people with African descendants compared to Indians and other races which showed a race dependant patern in PChE polymorphism. The obtained prevalence of heterozygous atypical in our study (1%), is also less than the figures reported for an Iranian sample with Jewish descent (20) and white Americans (about 5%) (15). The geographical pattern of genotypes polymorphism, which was frequently associated with racial differences, is a well known and documented fact. However, by exponentially increasing migration among the different populations since the last decades, these differences are going to be significantly faded away. The prevalence of some other genes polymorphism have been studied and well documented by other researchers in Mazandaran province. For example, the prevalence of some variants of G6PD deficiency has been reported to be higher in Mazandaran province compared to the other parts of the world (26). In most studies conducted in other parts of Iran, the frequency of atypical heterozygous genotype of PChE had shown minor differences with other societies across the world (**9**, 19). Since the frequency of homozygous genotype ($E_{a,a}$) is obviously correlated to the heterozygous genotype ($E_{a,u}$), according to our finding (1% frequency of $E_{a,u}$ genotype), the risk of postoperative apnea following the use of muscle relaxants (i.e. succynilcholine and mivacurium) in Mazandaran province is not higher than the other regions of the world including the other provinces in Iran.

Finally as a conclusion, we found out that the prevalence of pseudocholinesterase heterozygous atypical genotype in Mazandaran province (1%) is less than the values previously reported in other parts around the country. Hence, the prevalence of atypical homozygous phenotype ($E_{a,a}$) and consequently the risk of prolonged apnea during and after anesthesia (induced by defects in pseudocholinesterase) is much lesser than the other parts of Iran and some parts of the world.

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Conflict of Interest: There was no conflict of interest.

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