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RESEARCH ARTICLE

GENETIC POLYMORPHISM OF PARAOXONASE 3 GENE ALA99ALA IN *ADJA*AND*MAHI*ETHNIC GROUPS IN BENIN.

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Abstract

Introduction: The paraoxonase has anti-atherogenic activity which may be altered by the coding gene polymorphisms. The purpose of this study was to assess the genotypic distribution and allelic frequencies in paraoxonase 3 gene Ala99Ala polymorphism in Beninese *Adja*and *Mahi* ethnic groups.

Materials and methods: The paraoxonase 3 gene Ala99Ala polymorphism of 144 subjects of Beninese *Adja*and*Mahi* ethnic groups was studied using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique and compared with that in Beninese Abomey-Calavipopulationand others world populations.

Results: The alleles A and G frequencies were 67.0percent and 33.0 percent respectively of*Adja*ethnic group; and 66.0 percent and 34.0percentrespectively of*Mahi* ethnic group. There were no significant ethnic differences for these allelic frequencies between these two ethnic groups.

Conclusion: The genotypic distribution and allelic frequenciesof paraoxonase 3 gene Ala99Ala polymorphism in Beninese*Adja*and *Mahi* ethnic groups were significantly different from those in Beninese Abomey-Calavipopulationand in others world populations respectively.

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Introduction:-

The paraoxonases (PON) enzymes are associated with many inflammatory diseases, such as cardiovascular diseases, by protecting the cells again oxidative stress (Aviram and Rosenblat, 2004). They are associated with high-density lipoprotein (HDL), hydrolyzed lactones and inhibited the oxidation of low-density lipoprotein (LDL), a function that is believed to slow the initiation and progression of atherosclerosis (Seres et al, 2004 andZhang et al, 2010). The paraoxonase gene has three isoforms, PON1, PON2 and PON3 (Draganov et al, 2005) located on chromosome 7q21.3–22.1 and covers approximately 136kb (Primo-Parmo et al, 1996). Various populations' studies have reported inter-ethnic differences in the allele frequencies for PON gene polymorphisms (Campo et al, 2004 and Wu et al, 2010). This variability suggests that ethnic differences, gene-gene interactions and susceptibility to environmental factors might modulate the relationship between PON polymorphisms and many inflammatory diseases (Wang et al,

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2003). Many studies have been conducted on the relation between PON polymorphisms and genetic susceptibility to coronary heart disease (Erlich et al, 2006 and Zhang et al, 2013). The paraoxonase 3 (PON3) have a similar role to PON1 (Sanghera et al, 2008). The expression of PON3 gene occurs mainly in the liver and bind to HDL in blood stream (Reddy et al, 2001). The PON3 gene has five commons Single Nucleotide Polymorphisms (SNPs) Ala99Ala, Asp107Asn, Glu146Lys, Ala179Asp and Tyr233Cys (Robertson et al, 2003; Li et al, 2004; Pasdar et al, 2006 and Sanghera et al, 2008). Despite the importance of the paraoxonase gene and its implications on the genetic susceptibility to coronary heart disease, no studies on African black populations have been reported. The purpose of this study was to assess the distribution of PON3 gene Ala99Ala polymorphism in Beninese ethnic groups,*Adja* and *Mahi*in order to investigate the genetic basis of PON3 gene related diseases in Beninese population.

Materials and Methods:-

Subjects:-

This study has been conducted on two Beninese ethnic groups *Adja* and *Mahi*. In June 2015, fifty (50) unrelated volunteers 13 men and 37 women, aged between 17 and 72 years old; were selected randomly from *Adja*ethnic group from Lokossa, a south-west city in Benin; and in November 2015, ninety-four (94) volunteers,33 men and 61 women, aged between 6 and 70 years old; were selected randomly from *Mahi*ethnic group from Savalou, a central cityin Benin.

DNA extraction and genotyping:-

Venous blood samples were collected from each volunteer after written consent. Genomic DNA was extracted by phenol-chloroform method at Genetics and Biotechnologies Laboratory (GBL) at Abomey-Calavi University. The Polymerase Chain Reaction (PCR) primers sequences for the PON3 Ala99Ala SNP (rs1053275) were designed as previously described by Wu *et al*(2010) and synthesized by SANGON Biotech (Shanghai, China). The sequences were: forward 5'-TCCAGGCATGCCAAACTTT-3' and reverse 5'-TTTCCCTCATTTCCCCCTT-3' were used to amplify 197 bpfragment containing the polymorphism site Ala99Ala on PON3 gene. PCR was performed using thermocycler PTC 100™ (*Programmable Thermal Controller*; Perkin Elmer) in a final volume of 25 µL as follows: 3 µL (3 ng) DNA sample was added to a reaction mixture containing 2.5 µL of 10×PCR buffer, 1.5 µL of 25 mmol/L $MgCl_2$, 1µL of 200 µmol/L dNTPs, 1U Taq DNA polymerase (1 µL) (Fermentas) and 2 µL of 0.2 µmol/L of each primer, dimethyl sulfoxide (DMSO) was added to a final concentration of 5% and ultra-pure water (Merck) to a final volume of 25 µL. The fragment amplification was performed under the following conditions: 10 min pre-denaturation at 94 °C followed by 30 cycles of 45 sec denaturation at 94 °C, 45 sec hybridization at 50 °C and 45 sec extension at 72 °C; and finished by 7 min extension at 72 °C. Then 10 µL of PCR products was digested with 2 µL of 10xNEB buffer, 2 µL of HhaI endonuclease (Promega) and ultra-pure water to a final volume of 20 µL. Tubes were incubated at 37°C for 4h before separation on a 2percentagarose gel and visualization by staining in ethidium bromide under UV trans-illumination.Digestion recognition sequences were GCGC, thus the G allele version would be digested by the enzyme HhaI. The expected results for this polymorphism were the electrophoretic profile with 112 bp, 63 bp and 22 bp bands corresponded to the homozygote genotype G/G; 175 bp and 22 bp bands to the homozygote genotype A/A; and 175 bp, 112 bp, 63 bp and 22 bp bands to the heterozygote genotype G/A. The 63 bpand 22 bp bands were not visible on the agarose gel.

Statistical analysis:-

Alleles and genotypes frequencies were calculated by gene counting. The chi-square test on sphinx V 11 software was used both to estimate the Hardy-Weinberg equilibrium and to compare the allelic frequencies observed in Benin population with those reported in other world populations. Value of $p < 0.05$ was considered statistically significant.

Results:-

PON3 gene Ala99Ala polymorphism identification:-

The PON 3 gene Ala99Ala polymorphism (rs1053275) is a G to A substitution resulting a synonymous change, Ala (GCG) to Ala (GCA). One-hundred-forty-four (144) samples from Beninese *Adja*and*Mahi*ethnic groupswere used to amplify a 197 bp fragment, digested with HhaI. The figure 1 represents the electrophoretic profile of the different genotypes. The PCR products 197 bpfragmentwere represented on the gel by SG band. On the gel, the individuals A9, A12, M1 and M7 were genotyped G/A (with both fragment of 175 bp and 112 bp); the individualsA1, A3 and M2 weregenotyped A/A (with only one fragment of 175 bp) andthe individuals A19 and M22 were genotyped G/G (with only one fragment of 112 bp).

PON3 gene Ala99Ala polymorphism genotypes distribution and alleles frequencies:-
The genotypic and allelic frequencies of PON3 Ala99Ala polymorphism in Beninese *Adja* and *Mahi* ethnic groupswas shown in Table 1.Two alleles and three genotypes were observed in Beninese *Adja* and *Mahi* ethnic groups. The homozygote individual G/Gwasfound in these two ethnic groups. The observed and expected frequencies for the polymorphisms were at Hardy-Weinberg equilibrium ($p > 0.05$).

The comparison of PON3 A99A allelic frequencies within Beninese different ethnic groups was shown in Table 2. There were no significant ethnic differences between *Adja* and *Mahi*for PON3 gene Ala99Ala polymorphism allelic frequencies($p>0.05$).But significant differences were observed for PON3 gene Ala99Ala polymorphism allelic frequencies in these towethnic groups compared to Beninese Abomey-Calavi population respectively ($p<0.05$).

The comparison of PON3 gene Ala99Ala allelic frequencies in Beninese *Adja* and *Mahi*ethnic groups with those in other word populations was shown on Table 3. There were significant differences in PON3 Ala99Ala polymorphism allelic frequencies in Beninese *Adja*and*Mahi*ethnic groups compared to Chinese *Li* minority and British*Caucasians*.

Discussion:-

The present study has determined the genotypes distribution and the allelic frequencies of PON3 gene Ala99Ala polymorphism in Beninese *Adja*and*Mahi*ethnic groups. Two alleles and three genotypes were observed. These genotypic and allelic frequencies of PON3 gene Ala99Ala polymorphism in the Beninese *Adja*and*Mahi*ethnic groups were compared with previously described frequencies in Beninese Abomey-Calavi population and others world populations (Chineses and Britishs).

No significant ethnic differences in PON3 gene Ala99Ala polymorphism genotypes distribution between *Adja* and *Mah*i ethnic groups were observed. These two ethnic groups were referred as homogenous population in Benin, so may have the same characteristics in genetic information's transmission. However, there were significant differences in PON3 gene Ala99Ala polymorphism genotype distribution and alleles frequencies in Beninese *Adja*and*Mahi*ethnic groups compared to Beninese Abomey-Calavi population respectively (Segbo et al, 2014). In contrast with this previous study, the homozygote individual G/G was found in *Adja*and*Mahi* ethnic groups. Abomey-Calavi population, the major ethnic group is among the heterogeneous ethnic group; living in the south-central area (Abomey-Calavi city), the denser area in the country. While *Adja*and*Mahi*ethnic groups were considered asmuch homogenouspopulations within which the genetic traits transmission were much conservative. So the transmission of PON3 gene Ala99Ala polymorphism genotypes distribution in Beninese *Adja*and*Mahi*ethnic groups were much conservative and this polymorphism site may be used as an excellentgenetic marker for DNA analysis in Benin.

There were significant differences in PON3 Ala99Ala polymorphism genotype distribution and allelic frequencies in Beninese *Adja*and*Mahi*ethnic groupscompared to Chinese *Li* minority respectively (Wu et al, 2010). The G/G genotype frequency in *Adja*ethnic group (10.0 %) was similar to that observed in Chinese *Li* minority (8.0 %), but was significant different from that observed in*Adja*ethnic group (19.0 %).There were also significant differences in PON3 Ala99Ala polymorphism distribution in Beninese *Adja*and*Mahi*ethnic groups compared to British Caucasians ($p<0.05$)(Robertson, 2003; Pasdar, 2006).

Additional studies on large cohorts from different ethnic groups in Benin may be needed to determine the real genotypes distribution of PON3 Ala99Ala polymorphism in Beninese population

Conclusion:-

In this study, the genotypes distribution and allelic frequencies of the PON3 gene Ala99Ala polymorphism in Beninese *Adja*and*Mahi*ethnic groups were described and compared with other world populations.The PON3 gene Ala99Ala polymorphism distribution in Beninese *Adja*and*Mahi*ethnic groupswere significant different from other populations. These ethnic variations in PON3 gene polymorphisms can be used as basis for further investigation on the association of this polymorphism with the risk of cardiovascular diseases and other inflammatory diseases.

Table 1:- Genotypic and allelic frequenciesof PON3 gene Ala99Ala polymorphism in Beninese *Adja* and *Mahi*ethnic groups

Ethnic groups	n	Genotypes			Alleles	
		A/A	G/A	G/G	A	G
Adja	50	44.0	46.0	10.0	67.0	33.0
Mahi	94	51.0	30.0	19.0	66.0	34.0

Table 2:- Comparison of PON3 A99A allelic frequencieswithin Beninese different ethnic groups

	Adja	***Mahi***	***Fon***	***Adja*vs*Mahi***	***Fon*vs*Mahi***	***Fon*vs*Adja***
				pvalue	***pvalue***	***pvalue***
Alleles	33.0	34.0	2.0	$p > 0.05$	$p < 0.05$	$p < 0.05$

Table 3:- Comparison of PON3 A99A allelic frequenciesin Beninese *Adja* and *Mahi*ethnic groups with those in other world populations.

Ethnic groups	n	Allelic frequencies (%)		p *values*
		A	G	
Beninese *Adja*	50	67.0	33.0	p > 0.05
Beninese *Mahi*	94	66.0	34.0	p > 0.05*
Chinese *Li* [a]minority	150	75.0	25.0	p < 0.05**
British *Caucasians*[b]	450	52.0	48.0	p < 0.05**

*no significant ethnic difference was observed when compared toBeninese *Adja* ethnic group;
** significant differences were observed when compared to*Adja* and *Mahi*ethnic groups;
[a] Wu et al. 2010 ; [b]Pasdar et al. 2006

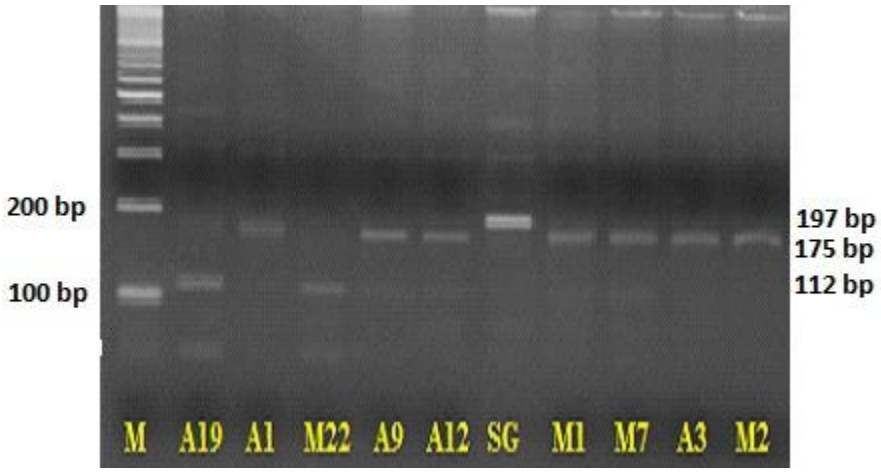

Figure 1:-Electrophoretic profile of the different genotypes (Segbo, et al).

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