

Genotyping of A251g, Copper-Zinc Superoxide Dismutase (CuZnSOD) Polymorphism, in Term Pregnant Women In Uyo, South-South, Nigeria

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Abstract: Cu/ZnSOD protein and its gene are located on chromosome 21q22. Cu/ZnSOD is found almost exclusively in intracellular cytoplasmic spaces and comprises 90% of total SOD making the investigation of Cu/ZnSOD expression levels has become very important. The A251G Copper-Zinc Superoxide Dismutase polymorphism is distributed widely among south -south pregnant Nigerian population, showing the prevalence of the wild type. Fifty apparently healthy subjects were recruited from St. Luke's Hospital Anua, Akwa-Ibom State, Nigeria during the antenatal clinic. Genotyping of A251G polymorphism was done using polymerase chain reaction (PCR) followed by restriction digestion (RFLP) using MspI(*Moraxella* species), an Isoschizomer of HpaII. The study shows 64.10 % were wild type, 25.64 % were heterozygous and 10.26 % were homozygous for A251G CuZnSOD polymorphism. The A allele frequency which was used as the reference allele was the most common, 75%, while the least common was the G allele, 25%, which was used as the variant. This study was prompted by the absence of published data on the prevalence of A251G CuZnSOD polymorphism in Nigeria.

Keywords: A251G Copper Zinc Superoxide Dismutase; PCR; RFLP, Genotyping

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I. Introduction

Cu/ZnSOD protein and its gene is located on chromosome 21q22. Cu/ZnSOD is found almost exclusively in intracellular cytoplasmic spaces and comprises 90 % of total SOD making the investigation of Cu/ZnSOD expression levels very important (Noor et al., 2002). It codes for the monomeric SOD1 polypeptide (153 amino acids, molecular weight 16 KDa). The coding region consists of five exons interrupted by four introns. Several polymorphisms have been identified in SOD1 gene, mainly distributed in the regulatory regions, including promoter and introns (Osredkar et al., 2011). It functions mainly in catalysing the dismutation of superoxide radical to hydrogen peroxide. Many mutations in SOD1 gene polymorphism has been implicated in conditions such as amyotrophic lateral sclerosis (Rosen et al., 1993), in breast cancer (Asaduzzaman Khan, Tania, Zhang and Chen, 2010) and cataract, and in Down's Syndrome. Little is documented about polymorphisms in oxidative stress related genotypes (enzymes involved in the scavenging of reactive oxygen species), and the frequency of these genotypes in Uyo, Akwa Ibom State. This study provides baseline data of the variations, frequency and prevalence in polymorphism of Copper/Zinc superoxide dismutase (Cu/ZnSOD) genotypes in term pregnant women in Uyo, within Akwa Ibom State of Nigeria.

II. Materials And Methods

Subjects

A total of 50 term pregnant women (7 to 9 months) unrelated, apparently healthy subjects from St. Lukes's Hospital Anua, Uyo, Akwa Ibom State were studied. At the time of blood sampling, most of the women investigated (39.06 %) were of age range 21 to 26 years, while the minority (10 %) of the women were of age range between 33 to 38 years of age. All subjects were fully informed about study protocol and have consented to participate in the study. The study was approved by the Ethical Committee of St. Luke's Hospital, Anua, Uyo, Akwa Ibom State.

III. Methods

The DNA was isolated from EDTA-whole blood, using using Qiagen® mini-blood kits according to the manufacturer's protocol (QIAGEN, GmbH, Hilden, Germany). The A251G Cu/ZnSOD polymorphism was genotyped by polymerase chain reaction as par manufacturer manual, followed by restriction digestion with 5U MspI restriction enzyme (PCRRFLP). An A>G in intron was amplified to form undigested fragments of 570 bp using primers: 5'AGTACTGTCAACCACTAGCA-3' and 5'-CCAGTGTGCGGCCAATGATG-3' as forward and reverse primers, respectively (Sinagene). The PCR mixture comprised of 13.95µl water, 2.5µl buffer10X, 0.75µl MgCl₂, 0.5µl dNTP, 1µl forward and reverse primers and 0.3µl Taq DNA polymerase for every 5µl DNA sample. PCR conditions were 94°C for 4 min, followed by 33 cycles of 94°C for 50 s, 63°C for 50 s, 72°C for 50 s, and a final extension step at 72°C for 7 min. The 570 bp PCR products were digested at 37 °C overnight with 5U MspI and separated on a 2 % agarose gel (Yousefnia, 2014). Wild type genotype AA is detected by the presence of a 570 bp fragment, and homozygous genotype GG characterized by the presence of two fragments of 375 and 201 bp for homozygous; three fragments of 570, 375, and 201 bp for heterozygous genotype (AG) (FIG.1).

IV. Statistical Analysis

The results were analysed using SPSS version 17, and Microsoft data tool pak, Excel 2007. Mean (M) and standard error of mean (S.E.M) were used as measures of the data distribution. The acceptable level of significance was set at P<0.05.

V. Results

Fifty apparently healthy term pregnant women attending antenatal at St, Luke's Hospital Anua, Uyo, Akwa Ibom State were tested for A251G Cu/ZnSOD polymorphism. Table 1 presents the descriptive data of the tested subjects based on their inherited genetic polymorphism. Maternal age for wildtype, heterozygous and homozygous type were 26.28, 27.00 and 25.14 years respectively. Parity for wildtype, heterozygous and homozygous type were 2.47, 2.42 and 2.57 respectively. Body Mass Index for wildtype, heterozygous and homozygous type were 24.52, 26.63, and 26.12 kg/m² respectively. Weight for wildtype, heterozygous and homozygous type were 66.66, 70.81 and 69.71kg respectively. Height for wildtype, heterozygous and homozygous type were 1.61, 1.57 and 1.64 meters respectively. Figure 1, shows the results of restriction analysis of SOD1 gene polymorphism, MspI digestion resulted in one fragment of 570 bp for wild-type (AA); two fragments of 375 and 201 bp for homozygous (GG); and three fragments of 570, 375, and 201 bp for heterozygous (AG).

In Figure 2, the analysis of the allele frequencies shows that in this study population A allele frequency was the most common (75 %), and was used as the reference genotype, while the less common allele was G allele (25 %) and was examined as the variant. The distribution of wild-type (AA), heterozygous type (AG) and homozygous type (GG) were 64.10, 25.64, 10.26 % respectively. The frequencies for wild type were the highest which compares with the report of Hong et al.(2002) in other population, while the homozygous type were the minority (10.26 %). This study did not find statistically significant differences in the percentage distributions of A251G Cu/ZnSOD polymorphism.

The frequency of normal A allele was 75% in subjects while the frequency of normal G allele was 25% in subjects (Table 2)



Figure 1: PCR-RFLP data after digestion by MspI on electrophoresis gel for SOD1 gene. Showing Wild type (AA) characterized by the presence of a 570 bp fragment, and homozygous genotype (GG) characterized by the presence of two fragments of 375 and 201 bp; three fragments of 570, 375, and 201 bp for heterozygous genotype (AG)

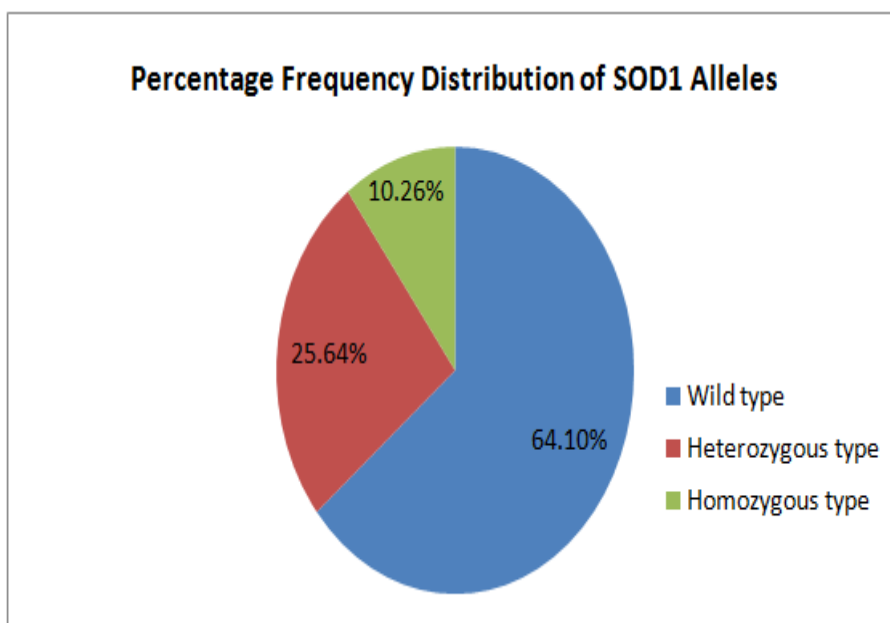


Figure 2: Distribution of SOD1 alleles for term pregnant women in St. Luke’s Hospital, Anua, Uyo, Akwa Ibom State, Nigeria.

Table 1 . Socio-demographic parameter of the different genotype groups of subjects.

| Parameters | Wild Type (N=32) | Heterozygous Type (N=12) | Homozygous Type (N=6) |
|-------------------------|------------------|--------------------------|-----------------------|
| Maternal age (yrs) | 26.28 ± 0.987 | 27.00 ± 1.225 | 25.14 ± 2.198 |
| Parity | 2.47 ± 0.277 | 2.42 ± 0.36 | 2.57 ± 0.481 |
| BMI(kg/m ²) | 24.52 ± 1.617 | 26.63 ± 3.06 | 26.12 ± 1.529 |
| Weight (kg) | 66.66 ± 1.718 | 70.81 ± 4.51 | 69.71 ± 3.695 |
| Height(m) | 1.61 ± 0.019 | 1.57 ± 0.385 | 1.64 ± 0.013 |

Table 2. The frequencies of A and G alleles among term pregnant women in St. Luke’s Hospital Anua, Uyo, Akwa Ibom State.

| Allele | Frequency | Percentage |
|----------|-----------|------------|
| A allele | 0.75 | 75 % |
| G allele | 0.25 | 25 % |

VI. Discussion

Cu-Zn SOD (SOD1) gene influences the concentration of superoxide dismutase enzyme produced in cytoplasm, which contributes to the neutralization of ROS and serves as a main line of defense against on-going oxidative stress. Cu is associated in the Cu/Zn SOD it functions as an antioxidant expressed in both maternal and fetal tissue. Studies have shown increased levels of Cu in placenta, maternal serum, and amniotic fluid in preeclamptic women compared to controls. Zn has antioxidant functions through the Cu/Zn SOD. Recent studies in Zn supplementation show reduced incidence of pregnancy induced hypertension and Zn deficiency has been associated with preeclampsia with decreased levels in placental tissue and maternal serum (Hansson et al., 2014).

Both genetic and biochemical studies carried out on SOD1 demonstrates that SOD1 gains importance in the development of diseases such as heart failure, cancer, diabetes, down’s syndrome, and amyotrophic lateral sclerosis. In addition to the disease implications, decreased activity of the antioxidant enzymes and depletion of total antioxidant capacity tends to increase susceptibility to oxidative injury (Nithya, Angeline, Isabel and Asirvatham, 2016).

Several studies have provided evidence that heritable polymorphisms including MnSOD polymorphism (SOD2) exist and could directly modify oxidative damage to varying degrees among Asian and Caucasian pregnant women (Hong, Lee, Yi, Ha, and Christiani, 2002). Data regarding the SOD1 gene polymorphism is available for some populations including North Indians, the Bangladeshi, the Finns, the Romanians, the New Zealanders and the Czechs, it is lacking among African population. There is little information about frequency or implications of Copper/ Zinc s (Vats, Sagar, Singh and Banerjee 2015). The literature review showed that DNA sequence changes in SOD1 gene can be detected in about 0.3% of humans worldwide, and hypothesized that mutations in the SOD1 gene may impair antioxidant enzyme activity thereby leading to accumulation of toxic superoxide anions .

Population genetics studies highlight geographical and ethnic differences which may suggest evolutionary pressures generated by environmental factors .This study provides a baseline survey of the frequency of Cu/ZnSOD polymorphism among term pregnant women in St. Luke's Hospital Anua, Uyo, Akwa Ibom State, Nigeria. This study will enable investigations on the impact of the named maternal genetic polymorphism on the level of oxidative stress in the context of the ethnicity represented in south-south Nigerian community. In Conclusion, in terms of the prevalence of specific SOD1 genes, our study shows that 64.10 % of the population has the wild type (AA) SOD1 gene, in this population 10.26 % of the women had the homozygous genotype and 25.64 % of this population had the heterozygous genotype. Wild type SOD1 genes were most prevalent in the population of women studied. This corresponds with literature reports showing prevalence of wildtype genotype in South Indian population (Nithya, Angeline, Isabel and Asirvatham, 2016).

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