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The Association between Polymorphism-119 G > A of the Alpha-Fetoprotein Gene and Increased Serum Alpha- Fetoprotein Level: A Case Control Study

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ABSTRACT

Background: Alpha-fetoprotein is produced during pregnancy by the yolk sac, and then by the fetal liver. This hormone is secreted from early pregnancy, but its level of measurement is important from 15 to 21 weeks of gestation. The secretion of this hormone is very important because an increase in this hormone is associated with neural tube defects (NTDs) in the fetus. In this study, we analyzed the association between polymorphism-119 G > A in the alpha-fetoprotein (*AFP*) gene with increased AFP levels in mothers with no neural tube defects in their fetus.

Methods: Participants were 50 pregnant women who had high alpha-fetoprotein but no neural tube defects in their fetus (the case group) and 50 pregnant women that their alpha fetoprotein was normal with no neural tube defects in their fetus (control group). After DNA extraction, the fragment including the desired polymorphism was amplified using the PCR technique, and then sequenced.

Results: The results of this study showed that there was no significant relationship between this polymorphism and the increase in alpha fetoprotein in the study population.

Conclusion: Future studies are needed to investigate other polymorphisms with higher specimens, and the relationship between this polymorphism, and the survival rate of pregnant mothers in the Iranian population.

Introduction

Neural tube formation is an embryonic process that can be divided into two stages. The first stage is done in three to four weeks of pregnancy. In this stage, the brain is formed and in the second stage, distal sacral regions are formed.

Disorders during this period lead to the neural tube not closing completely or partially in the fetus.[1,2] Impaired neural tube closure happens during embryonic period. These disorders occur in the first month of pregnancy. The nerve plate and its coverings fail to connect to each other properly about 27 days of pregnancy. The most frequent types are spina bifida and encephalocel. Positive family history increases the risk of NTDs.[3]

Alpha-fetoprotein is a peptidoglycoprotein composed of 591 amino acids and has a molecular weight of 70,000 Daltons, which is genetically and structurally similar to human albumin. Both proteins are encoded by a gene on chromosome 4. This protein is produced during the pregnancy by the yolk sac, and then by the fetal liver. This hormone is secreted since early pregnancy, but its serum level is important from 15 to 21 weeks of gestation.[4] The secretion of this hormone is very important because an increase in this hormone is associated with neural tube defects in the fetus. In some pregnant women, the level of this hormone rises, but their anomaly sonography is normal, and there is no defect in the neural tube of their fetus.[5] Serum AFP level measurement is a part of the pregnancy screening tests. AFP reaches its maximum level up to one-third of fetal serum proteins in the second trimester of pregnancy.[6] Liver production of AFP is almost constant until 30 weeks of gestation and then it decreases.[4]

A severely enhanced Multiple of the Median (MoM) of > 1.7 is also considered as strong hint on sonographically detectable fetal malformations.[7]

The AFP -119 G > A (rs587776861) polymorphism had previously been studied in a population based study which there was no significant association between elevated AFP levels and NTDs. The present study investigated the association between the polymorphism -119 G > A at the AFP gene and NTDs in the Iranian pregnant mothers.

Materials and Methods

Patients: We enrolled 50 pregnant women with an average age of 29.8 ± 5.98 years who had high AFP (the case group) and 50 pregnant women with an average age of 31.4 ± 4.28 years that their AFP was normal (control group) referred to Dr. Mazaheri Medical Genetics Center, Yazd, Iran. None of them had NTDs in their fetus. The mean level of alpha fetoprotein was 1.1 ± 0.43 MOM and 3.3 ± 0.59 MOM in case and control group, respectively.

Genetic analysis of AFP polymorphism: After justifying and ensuring the confidentiality of the information and obtaining written consent, for analyzing the AFP polymorphisms, genomic DNA was isolated from blood extracted DNA by FavorPrep Blood Genomic DNA Extraction Mini Kit and stored at -20°C. Two primers were used for genetic screening of polymorphisms in the AFP. The primers of the AFP gene, F1-AFP, and R1-AFP (Table 1) were then designed with Pre-primer software as well as the UCSC website. The specificity of the primers was assayed by 'BLAST' program at http://www.ncbi.nlm.nih.gov/blast.

PCR was performed in a total volume of 50 mL containing 150 ng of template DNA, 10 pmol of each primers12.5µl PCR Master Mix (Yekta Tajhiz Azma Co., Tehran, Iran).

Table 1. Primers used for AFP gene, polymorphism -119 G > A

Number	Name	Sequence (5'→3')	Length
1	F1-*AFP*	GATGAAGAGTCTGAATTGGT	20
2	R1-*AFP*	TGAGGGAGTTATGTGAAGG	19

PCR amplification was carried out at 95°C for 5 min, followed by denaturation at 95°C for 1min, annealing at 60°C 1 min and extension at 72°C for 1 min (32 cycles) followed by a final extension for 5 min. The PCR products were electrophoresed on a 1.5% Agarose gel (Figure 1). PCR products were purified with ExoSAP followed by bidirectional sequencing with the BigDye Terminator v1.1 Cycle Sequencing kit on an ABI3130XL Genetic Analyzer.

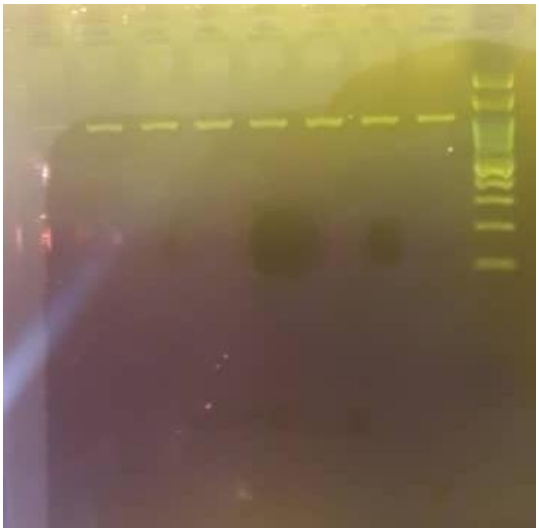

Figure 1. PCR product electrophoresis image examples

Results

In this case-control study, we evaluated the polymorphism -119 G > A in the *AFP* gene in 50 pregnant women with high alpha-fetoprotein levels without NTDs in their fetus.

The allelic frequencies of *AFP* gene polymorphisms in patients and healthy women are shown in Table 2.

Table 2. Comparison table of allele frequencies

Name	G Allel	A Allel	Total	Significant relationship
Case	100	0	100	P value > 0.05
Control	100	0	100	

The participants were investigated for -119G > A with Sanger sequencing. The sequences were analyzed with the FinchTV_1_4_0 software (Figure 2).

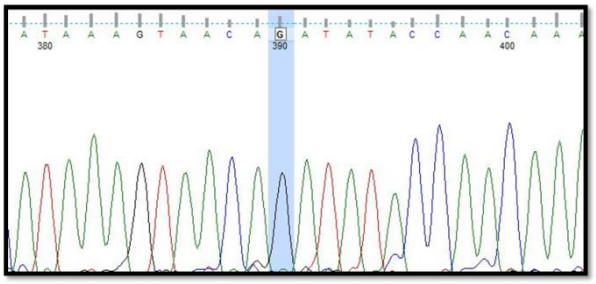

Figure 2. Part of the sequence of blue samples examined is the possible area of mutation

The result showed that none of the mothers in case and control groups had the mutant allele. Moreover, there was no significant association between the polymorphism and an increased level of alpha fetoprotein in the female population.

Discussion

In this case-control study, polymorphism -119 G > A in the alpha-fetoprotein gene was studied in 50 pregnant women who had high serum levels of alpha-fetoprotein and 50 pregnant women with normal levels of alpha-fetoprotein. Studies have shown that in the second trimester screening for a pregnant woman (i.e. 15 weeks), if serum alpha-fetoprotein level was high, the woman would be in the high-risk group and her fetus might have a neural tube defect.

The *AFP* gene is located on chromosome 4 in q11-13 region.[8] Regarding AFP, the upstream of the 5 *AFP* gene is very important in gene transcription regulation because it contains three elements (amplifiers, promoters, and insulator) that regulate the exact transcription of the *AFP* gene. Two transcription factors involved in regulating the *AFP* gene are hepatic nuclear factor (HNF-1) and non-tissue factor (NF-1).[9] HNF-1 activates and stimulates the *AFP* gene.[10] However, NF-1 in high concentrations of AFP suppresses gene activity and increases AFP levels in low concentrations.[11] Studies have shown that there are two specific points in the HNF-1 binding sites of the *AFP* gene promoter where the mutations increase gene transcription and AFP levels. These mutations include -55 C > A at the proximal HNF-

1junction and an -119 G > A substitution at the HNF-1 distal junction.[12] Mutations in this site increase HNF-1 binding, resulting in increased transcription of the *AFP* gene and increased AFP levels. Interestingly, the HNF-1 site partially overlaps the detection site for the NF-1.[13] Therefore the increase in the HNF-1 will reduce NF-1 binding and increase AFP levels. In short, mutations in two points increase *AFP* gene transcription and an increase in AFP levels.[9]

Studies show that there is little evidence on the increase in alpha fetoprotein. Genetics and different disorders may increase AFP levels. Non-hepatic disorders and hepatic disorders can increase AFP levels. In benign breast disease, peptic ulcer disease and chronic lung disease, liver cirrhosis, acute and chronic hepatitis, and drug-induced liver damage, serum AFP levels may also increase due to hepatic regeneration, as the AFP is synthesized by the growing liver.[14] Various studies have shown that serum AFP level increases in malignancies such as hepatoblastoma, testicular cancer, patients with germ cell tumors, pancreatic cancer, gastric cancer, colorectal cancer, lung cancer, and patients with hepatic carcinoma.

Several studies have shown that urinary disorders are among the conditions that can increase AFP levels [14]. The reason of such a wide variety of serum AFP levels is still unknown. Some of the known etiological causes of elevated maternal serum alpha-fetoprotein include: errors in calculating gestational age, multiple pregnancies, fetal anterior abdominal wall defects, intrauterine fetal death (IUFD), renal anomalies and NTDs.[14]

Conclusion

In summary, the results of this study showed that there was no significant relationship between this polymorphism and the increase in alpha fetoprotein in the study population. Further studies are needed to investigate other polymorphisms with higher specimens, and the relationship between this polymorphism, and the survival rate of pregnant mothers in the Iranian population.

Conflict of Interests

Authors have no conflict of interests.

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