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Association of *TaqI* Polymorphism of Vitamin D Receptor (VDR) Gene with Anemia in Saudi Women

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Abstract
Objectives: This study aims to correlate occurrence of anemia in a population of Saudi women with polymorphisms in the VDR gene.
Methods: The study sample consisted of 50 anemic women with vitamin D deficiency and 50 healthy women with normal hemoglobin and sufficient serum vitamin D levels. Restriction fragment length polymorphism (RFLP) analysis of the VDR genes was done for Single Nucleotide Polymorphism (SNP) sites namely *ApaI*, *BsmI* and *TaqI*. Statistical correlation was done between gene polymorphisms at these three sites and hemoglobin levels.
Results: We found a significant association between *TaqI* site polymorphisms of the VDR gene and presence of anemia in the study population.
Conclusion: This is the first report of a significant association between vitamin D receptor gene polymorphisms and anemia in saudi population. Further studies on a larger population size will pave way to elucidation of the mechanism in which VDR gene polymorphisms exert an influence on anemia.
Keywords: VDR polymorphism, anemia, *TaqI* polymorphism, anemia, vitamin D deficiency

Introduction

Fat soluble vitamin D is required by the body for normal bone development and maintenance. It acts by increasing absorption of phosphate, calcium and magnesium. Vitamin D deficiency is implicated in a wide range of diseases, most notably in relation to bones.[1] Vitamin D deficiency is a health concern worldwide. Recent studies have shown vitamin D deficiency to play a major role in many metabolic as well as physiological disorders such as diabetes, cardiovascular disease, cancer and thyroid disorders.[2] Sunlight accounts for about 50 to 90% of supply of vitamin D.[3] Vitamin D regulates bone turnover by the stimulation of osteoclastic and osteoblastic cells.[4]

Variations of the VDR gene, that is present in chromosome 12, have been associated with various disorders and the polymorphisms vary to a great extent among different populations of the world.[5] Four frequently occurring single nucleotide polymorphisms (SNPs) namely: *ApaI*, *BsmI*, *FokI*, and *TaqI*, found at the 3′ end of the VDR gene have been studied in detail.[6,7] The VDR suppresses activation of T cells, therefore, T-cell mediated autoimmune diseases are associated with polymorphisms in this receptor gene.[8] Polymorphisms in the VDR gene have been extensively studied.[9]

Deficiency of iron leads to anemia. There are various variants of anemia, such as pernicious anemia, nutritional anemia, sickle cell anemia and hemolytic anemia. While some anemias are genetic, the most common form observed in a large section of the population worldwide is due to iron deficiency, in turn manifested as low levels of hemoglobin. According to a WHO report in 2008, 1;62 billion of the world population is anemic. In females, the main cause of anemia is blood loss during menstruation and pregnancy. Since nutritional deficiency is the most common cause of iron deficiency anemia, this study is aimed at studying the possible association between anemic, vitamin D deficient women with VDR gene polymorphism at three restriction sites *ApaI*, *TaqI* and *BsmI*. We found a significant association between the *TaqI* polymorphisms and occurrence of anemia in the study population.

Materials and Methods

Study Subjects

The study involved volunteers: 50 women in the age group 25–35 who were anemic and had low serum vitamin D levels. The control group consisted of 50 women in age group 25–35 who had sufficient blood hemoglobin and serum vitamin D levels. Care was taken to only include women in both groups who did not have any other health conditions such as diabetes, hypertension, cardiac disorders, thyroid disorders etc. Informed consent of the volunteers was obtained. Hemoglobin levels of all the volunteers were measured using CBC. Women who had hemoglobin levels lesser than 10 grams/dL were classified as anemic, while those with hemoglobin levels greater than 10 grams/dL were classified as healthy. Serum vitamin D levels were measured by blood test for 25-hydroxyvitamin D: 25(OH)D. Levels of less than 20 ng/mL are considered deficient, 20–30 ng/mL are considered as insufficient and levels greater than 30 ng/mL are considered sufficient.

Extraction of DNA and Amplification of Vitamin D Receptor Gene

The protocol followed is the same as the one in our previous study.[9] Genomic DNA was extracted from whole blood using QIAamp DNA blood mini kit (QIAGEN, USA, Cat. no.51104). The extracted DNA was stored at −20˚C for further PCR reactions (Table 1). Nanodrop2000c instrument from Thermo Scientific (USA) was used to calculate

concentration and purity of the extracted DNA (Table 2). The polymerase chain reaction (PCR) was carried out as described in our previous study.[9]

PCR Amplification

The amplification products were resolved on 1% agarose gels and 1X of Tris-borate-EDTA (TBE) buffer followed by staining with ethidium bromide and visualized under uv light.

Genotyping of the VDR Gene

After its amplification, PCR product of ~2229 bp was exposed to enzymatic restriction digestion by three enzymes which were purchased from Thermo Scientific: *ApaI*, *BsmI*, and *TaqI* (Figure 1). The conditions for restriction digestion were followed according to Iyer et al., 2017. The digested DNA fragments and the DNA size marker were segregated by electrophoresis on a 1% agarose gel stained with ethidium bromide and 1X TBE electrophoresis buffer.

Table 1. **Represents the PCR reaction mixture**

10–30 ng DNA template
2X reaction buffer
4 mMMg+2
4 µM dNTPs
0.2 µM each of forward and reverse primers
0.45 U taq DNA polymerase
Final volume made up with nuclease free water to 50 µl

Table 2. **Represents the PCR conditions for 30 cycles**[6]

Denaturation	Annealing	Extension	Final extension	Hold
95˚C for 30 sec	60˚C for 1 min	68˚C for 2 min	72˚C for 5 min	4˚C∞

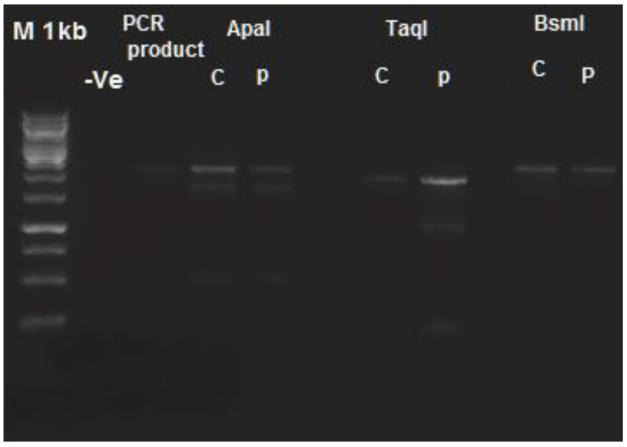

Fig. 1 **Agarose gel (1%) showing the results of PCR-RFLP comparison between the three restriction enzymes in anemia (P) and the control (C). Lane M: DNA marker. Lane 2: negative control. Lane 3: PCR product of size 2229 bp. Lane 4, 5: RFLP of *ApaI* digestion in control and patient. Lane 7, 8: RFLP of *TaqI* digestion in control and patient. Lane 10, 11: RFLP of *BsmI* digestion in control and patient.**

Results

PCR-RFLP Analyses and Distribution of the Genotypes

The restriction profiles obtained after PCR-RFLP are summarized in Table 3.

The distribution of various alleles in control group and anemic group are presented in Tables 4 and 5.

From Table 4, we observe that in the control group which comprised of healthy women with sufficient hemoglobin and serum vitamin D levels, there is no significant difference between the genotype frequencies in the Single nucleotide polymorphisms (SNPs) at any of the three sites. In contrast, as seen in Table 5, the study group of women with anemia and vitamin D deficiency clearly show a significant difference in

Table 3. **PCR – RFLP products for the restriction sites**

ApaI	*BsmI*	*TaqI*
AA – 2229 bp Aa – 2229 bp, 1700 bp and 529 bp aa – 1700 bp, 259 bp	BB - 2229 bp Bb – 2229 bp, 1579 bp, and 650 bp bb – 1579 bp and 650 bp	TT - 1982 bp and 247 bp Tt – 1982 bp, 1780 bp, 202 bp, and 247 bp tt – 1780 bp, 202 bp, and 247 bp

Table 4. **Distribution of various alleles in healthy women (sufficient hemoglobin and vitamin D levels)**

Restriction site	Genotype	Number of samples	% Frequency
ApaI	AA Aa aa	18 21 11 (total 50)	36 42 22 (total 100%)
BsmI	BB Bb bb	20 17 13 (total 50)	40 34 26 (total 100%)
TaqI	TT Tt tt	22 18 10 (total 50)	44 36 20 (total 100%)

Table 5. **Distribution of various alleles in women who were anemic as well as deficient in vitamin D**

Restriction site	Genotype	Number of samples	% Frequency
ApaI	AA Aa aa	19 21 10 (total 50)	38 42 20 (total 100%)
BsmI	BB Bb bb	22 16 12 (total 50)	44 32 24 (total 100%)
TaqI	TT Tt tt	9 10 **31** (total 50)	18 20 **62** (total 100%)

genotype frequency at the *TaqI* site. The genotype frequency of homozygous recessive alleles aa is 62% compared to TT and Tt genotypes, while in control subjects the homozygous recessive genotype tt is observed in only 10% of the study subjects.

Discussion

In recent times, anemia has been recognized as a world wide health issue. Nutritional as well as metabolic disorders have been attributed to low hemoglobin levels associated with anemia. Although we are well aware of the role of vitamin D in bone formation, metabolic regulation and overall growth and development, studies over the past few years have categorically suggested the role of vitamin D in erythropoiesis.[10] Studies by Sim et al., 2010.,[11] have shown significant association between vitamin D deficiency and anemia. Cusato et al.,[12] 2015 proved the role of VDR gene polymorphisms in the ribavirin-induced anemia in HCV-patients at 2 and 4 weeks of medication. The VDR gene polymorphisms could attribute to significant receptor dysfunction thereby causing various disorders such as low bone mineral density, autoimmunity, infections, cardiovascular disease and cancers.[13] Moreover, studies on VDR gene polymorphism in various populations around the world have pointed out the significant role of the VDR gene in vitamin D deficiency.[14] Our own research group has established association of *BsmI* polymorphisms with type I diabetes, *ApaI* polymorphisms with type II diabetes and *TaqI* polymorphisms with gestational diabetes in Saudi population.[9] These findings encouraged us to probe the possible relation between anemia and VDR gene polymorphisms. With this as the premise, our current study was designed to look for a possible correlation between anemia and vitamin D deficiency with polymorphisms of the VDR gene at three SNP sites namely *ApaI*, *TaqI* and *BsmI*. We have taken extreme care to include only those subjects who were anemic as well as had low serum vitamin D levels as the study group while the control group were women with sufficient levels of hemoglobin as well as serum vitamin D. We have clearly observed that at the *TaqI* site, there is a significant difference in genotype frequency between study group and control group. The homozygous recessive genotype tt was observed in 62% of the study group as against mere 20% in the control group. A study by Yassin et al.,[15] in an Egyptian population showed no correlation between anemia and VDR polymorphism at *ApaI* and *TaqI* sites. Another study by Yu et al.,[16] showed a significant association between VDR polymorphisms and Aplastic anemia in a Chinese population. According to Erturk et al.,[17] *BsmI* polymorphism may have a role in anemia in hemodialysis patients with BB genotype being strongly associated with lower hemoglobin levels. To the best of our knowledge, our study is a pioneering finding that strongly establishes the association of homozygous recessive alleles tt of *TaqI* polymorphism (rs731236) SNP with anemia. The study is a very strong support for this finding because all the patients recruited for the study were anemic as well as vitamin D deficient, while the control group was sufficient in both hemoglobin as well as serum vitamin D level, clearly establishing the connection between the two deficiencies at the genotypic level. Medrano et al.,[18] previously have proved a role of calcitrol, the active form of vitamin D in hematopoiesis. This strongly supports our observation that links anemia and vitamin D anemia.

Conclusion

Our study is the first of its kind reporting a genotypic association between anemia and vitamin D deficiency with the homozygous tt genotype of the *TaqI* SNP showing significant association in the study group as compared to healthy control group. Our study was conducted in women in the age group of 25–35, who are in active menstruation and childbirth age. It would be interesting to extend the study to a larger population, such as post menopausal women as well as children and observe any genotypic variation.

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Disclosure Statement

The author declares no conflict of interest.

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