Original article

Study Of Vitamin D Receptor Gene Polymorphism And Its Association With Vitamin D Levels In Healthy Individuals.

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INTRODUCTION

To the medical researchers all over the globe Vitamin D had become topic of interest. As evidenced by recent research the world is facing the problem of vitamin D deficiency pandemic. Deficiency of vitamin D is a risk factor in humans, right from conception and throughout lifespan. It is one of the common medical condition undiagnosed and under treated nutritional deficiency in the world. Globally about one billion people are known to have vitamin D insufficiency or deficiency[1]. In general population worldwide the prevalence of vitamin D deficiency is about 70-100%. In India the increased prevalence of about 50-100% is due to low intake of dietary calcium[2].

The medical fraternities across the world are curious in realizing that vitamin D plays a major role in health and disease. It causes skeletal as well as extra skeletal manifestations. Vitamin D not only regulates calcium and phosphorus homeostasis but also protects the individuals from many diseases like malignancies, chronic infections, and cardiac problems and reduce the risk autoimmune diseases. The study is to impress upon the physicians about the gravity of the vitamin D deficiency problem throughout India and to make appropriate diagnosis and treatment with care and caution[3]. As the vitamin D status is improved worldwide it would have remarkable effects on public health and decrease the healthcare expenses for several chronic diseases.

Vitamin D exerts its action in the body by binding to vitamin D receptor(VDR) which is a member of nuclear hormone receptor super family. It modulates the transcription of target genes by complexing with vitamin D responsive elements (VDRES) in the promoter region of target genes[4]. Excellent opportunities are provided by genetic studies to relate molecular insights with epidemiological data and it gained much interest. Polymorphism referred as variations in the DNA sequence or gene which occur in at least 1% of population and can have only modest and subtle effects[5].

Many polymorphisms are known to exist in VDR gene according to recent studies, but the influence of this polymorphism on VDR protein function is not known. At the 3'end of the VDR gene three restriction fragments length polymorphisms (RFLP) for BsmI, ApaI and TaqI have been studied so far.

VDR gene polymorphisms could potentially influence the binding of 1,25(OH)2 D and the anti-proliferative effects of vitamin. Many studies have been reported on VDR polymorphism and its influence on different disease throughout the world on the contrary in our country there reports are minimal.

The TaqI polymorphism is a T/C nucleotide substitution (ATT to ATC) leading to a silent change at codon 352 (isoleucine) in exon IX[6].

Therefore this study is to find the frequency of VDR (Taq1) gene polymorphism by PCR based restriction analysis of normal individuals and to determine the association of this polymorphism with 25-hydroxy vitamin D levels.
Materials And Methods

This study was conducted in the Department of Biochemistry, Kilpauk Medical College Hospital, Chennai. All procedures concerning human subjects or patients were permitted by the Institutional Ethical Committee. Explicit written consent was obtained from the study population. The study population comprised of normal individuals coming for master health check up Kilpauk Medical College Hospital, Chennai. Known smokers, alcoholics, diabetes, hypertensives, coronary artery disease were excluded from the study.

For the study, 2 ml of 12 hours Fasting Venous Blood was collected under sterile conditions from the ante cubital vein. Serum was separated after centrifugation at 3000 rpm for 10 minutes, alliquoted, and stored at -20°C and were not thawed until the batch was analyzed for 25-hydroxy vitamin-D. 25-OH vitamin D in serum is determined using ADVIA CENTAUR vitamin D assay on ADVIA CENTAUR XP systems. The reference range of VIT D: 10–30 ng/ml.

For gene polymorphic studies fasting blood was collected in 2 ml EDTA coated and DNA extraction done using mini preparation kit from Helini biomolecules, Chennai. Extracted DNA was identified by 1% agarose gel electrophoresis and comparison with a known molecular weight 1kb DNA (Lambda DNA) ladder. PCR Master mix was used in the following composition of Tris HCl - pH 8.5, (NH4)2SO4, MgCl2 – 3 mM acts as catalyst and 0.2% Tween 20. dNTP’s were used in a concentration of 0.4 mM each. Taq polymerase in a concentration of 0.2 U/µl. Primers were used in a concentration of 10 pmol. PCR was carried out in a reaction in volume of 50µL with the following components in the following manner, 25 µL reconstituted PCR master mix (contains gel loading dye), 10 µL of reconstituted primers, 5.0 µL of DNA and 10 µL of distilled water.

Amplification of the extracted DNA was carried out in CYBERLAB SMART PCR-PRO thermal cycler with the following cycling conditions of Initial denaturation for 95°C /5min, 34cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 45 sec and final extension at 72°C for 5 min. Amplicons of 600 bp was identified by 2.5% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

Taq1 gene polymorphism at the site was spotted by digestion of amplified PCR product with taq1 restriction enzyme using 5 µL of Taq1 buffer (1x), 2.5µl Taq1 enzyme, 30.0 µL of PCR Product and 12.5µl of distilled water. The entire procedure was carried out in ice. The contents were mixed thoroughly. The eppendorf was then placed in a 65°C waterbath for 30 minute. Restriction digested product was subjected to 2.5% agarose gel electrophoresis for genotyping. Two restriction site for Taq1 within 600bp

Tt (homozygous) - yield 660bp, 400bp, 200bp,
Tt (heterozygous) - yield 660bp, 400bp, 200bp,
TT (homozygous) - yield 600bp

This table shows the genotype distribution among the study population of which the common genotype is homozygous tt genotype which accounts for (94%) and the heterozygous Tt genotype accounts for (6%). There is no homozygous TT genotype in our study population.
Table-2: Correlation between the genotype and vitamin D level in study population:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group 1 N (%)</th>
<th>Group 2 N (%)</th>
<th>Group 3 N (%)</th>
<th>Total N (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tt</td>
<td>21 (22.3%)</td>
<td>39 (41.5%)</td>
<td>34 (36.2%)</td>
<td>94 (100%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Tt</td>
<td>0 (0%)</td>
<td>4 (6.7%)</td>
<td>2 (33.3%)</td>
<td>6 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

This shows among the study population, the group-1 individuals have increased frequency of tt genotype of (22.3%) whereas the group-2 individuals have increased frequency of Tt genotype of (66.7%).

Discussion:
The present study is an attempt to evaluate the normal distribution of Taq1 gene polymorphism in unrelated normal healthy individuals. Of several allelic variants in the VDR gene, one which is present in 3'UTR is Taq1 gene polymorphism. It is a T/C nucleotide substitution (ATT to ATC) leading to a silent change at codon 352 both coding for isoleucine and is in linkage disequilibrium with other variants al[7].

The distribution of tt genotype is significantly higher among the study population. In this study population, we found 94% of tt genotype, 6% of Tt genotype and the homozygous TT genotype was not found. The presence of Taq1 ‘T’ allele in Asians is (8%) compared to Caucasians (43%) and Africans (31%) as stated in Kim Kostner et al[8].

In this study the frequency of T allele is 6%. The finding of this study is similar to a study done in Austria (Ewald et al) where the frequency of tt genotype was found higher than TT genotype[9]. The genotype distribution in this study is not similar compared to the studies done so far in Japan (Tokita et al,1996),china(kung et al 1996) American black Pennslyvania(zumudaetal,1997),North India (Hemant K Bid et al 2005). The genotype distribution was not found to be in agreement with Hardy Weinberg equilibrium in our study. Zemuza et al in his study describes that distribution of VDR genotypes was not in Hardy Weinberg equilibrium, the reason behind this may be, apart from genotyping errors, they may be due to chance fluctuations due to small samples, nonrandom mating, and migration into or out of the population, selective survivorship among genotypes, population stratification, and admixture of different ethnic group[10].

Hemant K Bid et al in their studies say that there was a lack of data regarding the allelic variations in taq1 genotype from Indian subcontinent where population ethnicities are quite common.

The other hypotheses which focus influence of polymorphism on the vitamin D status showed that Taq1 polymorphism does not influence vitamin D status. Further attempt should be mandatory in order to understand the molecular and cellular variations affected by the polymorphism and to execute observational studies in larger populations. Study of different haplotypes, instead of SNPs could reduce the inconsistencies found so far, until then the role of VDR polymorphism will still be a topic for debate.

Conclusion:
From this study we conclude that the predominant genotype in south Indian population is tt genotype. Also the homozygous genotype (TT) was not obtained in our study population. About 21% of individuals are vitamin D deficient.

Compliance with ethical standards:
Conflict of interest: Authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of Institution.

Source of support: Nil

REFERENCES