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Original Article

Genetics Section

Influence of Vitamin D Status and IL-10 Gene Promoter Polymorphism (rs1800871) on Plasma IL-10 Levels in Apparently Healthy Individuals from Southern India: A Cross-sectional Study

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ABSTRACT

Introduction: Interleukin-10 (IL-10) is a major anti-inflammatory cytokine, which plays an important role in regulating inflammatory responses of the immune system. Changes in IL-10 level and its function can cause an imbalance in immune response, which can be associated with various disease conditions.

Aim: To study the influence of vitamin D status and IL-10 gene promoter polymorphism (rs1800871) on circulating IL-10 cytokine levels in apparently healthy South Indian population.

Materials and Methods: A cross-sectional study was conducted at the Department of Clinical Pharmacology, JIPMER, Puducherry, India from January 2016 to December 2017. Apparently healthy South Indian volunteers (N=101) of either sex, age more than 18 years, were recruited for the study, after obtaining written informed consent. Serum 25-hydroxy vitamin D and plasma IL-10 levels were measured by using Chemiluminescence and Enzyme Linked Immunosorbent Assay (ELISA), respectively. IL-10 rs1800871 genotyping was performed by Real Time-polymerase Chain Reaction (RT-PCR). Values were expressed as median, Inter-quartile Range (IQR) and proportions were described as number with percentage.

Results: The median serum vitamin D and plasma IL-10 levels observed among the study population were 18.21 ng/mL IQR, 11.3-23.08 ng/mL) and 9.04pg/mL (IQR, 7.75, 11.34 pg/mL) respectively. The genotype and allele frequencies of rs1800871 were consistent with that of the African, South Asian population of 1000 genome project. Plasma IL-10 levels were not significantly different across genotypes (p-value=0.091), even though the median level among homozygous mutant (TT) volunteers was observed to be less (8.35 pg/mL vs 9.69 pg/mL, 9.83 pg/mL). The correlation between vitamin D and IL-10 levels was observed to be insignificant (p-value=0.143).

Conclusion: The present study has reported the rs1800871 genotype frequency, circulating serum vitamin D levels and plasma IL-10 levels in the apparently healthy South Indian population. IL-10 cytokine levels were not significant across the different genotype and vitamin D status groups. No significant correlation was observed between the IL-10 and vitamin D levels among the sample studied.

Keywords: Anti-inflammatory cytokine, Immunogenetics, Vitamin D

INTRODUCTION

Interleukins (IL) are the cytokines secreted by leucocytes, and act as a mode of communication between leucocytes to perform their role in immunological defense. IL-4, IL-10, IL-11, and IL-13 are the major anti-inflammatory cytokines. Among them, IL-10 is a potent anti-inflammatory cytokine which suppresses proinflammatory cytokines, encourages anti-inflammatory mechanisms, and maintains a balance [1]. IL-10 is also known as Cytokine Synthesis Inhibitory Factor (CSIF), because of its ability to inhibit production of proinflammatory cytokines from helper T (Th1) cells. IL-10 has a major role to play in regulating inflammatory responses of the immune system [2]. Macrophages, Dendritic Cells (DC), Th2 cells, neutrophils, Th17 cells, regulatory T (TReg) cells, natural killer cells to some extent, and even Th1 cells are involved in IL-10 production. IL-10 secretion depends on many factors including the type of antigen stimulus, range of cells involved in production at that time, as well as complex regulation of the immune system [2]. Alteration in IL-10 level and function can cause an imbalance in immune response, which can lead to various disease conditions. TReg cells by producing IL-10 cytokine can induce tolerance to repeated antigen exposure, and limit further damage [3].

As many as, 23 Single Nucleotide Polymorphisms (SNP) were observed in IL-10 gene promoter region [4]. These genetic factors could explain 50%-70% of the differences in IL-10 expression. These polymorphisms can increase or decrease the binding of transcriptional regulators to the region and lead to the enhanced or reduced expression of IL-10 [4]. IL-10 gene is located on long arm of chromosome 1 (q31-32). The site is related to the genes carrying a high risk for autoimmune diseases including Systemic Lupus Erythematosus (SLE). Three SNPs in the promoter region are studied in detail, which are -1082 G>A (rs1800896), -819 C>T (rs1800871), and -592 C>A (rs1800872). Among them, later two are in linkage disequilibrium and inherited together [5,6]. Chen TK et al., have observed that, the rs1800896, as well as, rs1800872 polymorphisms, were significant in determining the IL-10 cytokine levels in peripheral circulation [7].

The production of vitamin D within the human body utilising the sunlight as resource, redefines and redirects its role in health. Now, it has been considered as a prohormone which gets activated in the human body to active hormone and exerts its effect on various organ systems outside the organ of its production. Because of its function, now, vitamin D is being considered as an endocrine system [8].

Apart from its active role in calcium homeostasis, vitamin D plays a vital role in both innate, as well as, adaptive immunity. It is involved in the production of peptides, such as cathelicidins and β-defensins against invading pathogens. Vitamin D helps in switching the immune response from Th1 to Th2, and reduces damage occur due to excess immune activity. This process is done by suppressing Th1 response and reducing the production of proinflammatory cytokines, as well as, by enhancing Th2 response, T regulatory cells and anti-inflammatory cytokine synthesis [9].

The relationship between vitamin D and anti-inflammatory cytokine IL-10 has been understood based on the findings from in-vitro, experimental, and clinical studies [10,11]. In an in-vitro study, done on human epidermal cell lines, the active form of vitamin D (1, 25 dihydroxy vitamin D3) and synthetic vitamin D analogue calcipotriol induced the expression of IL-10 receptor gene to about 10-fold and 12-fold, respectively. The present study was undertaken in the backdrop of analysing the anti-psoriatic action of vitamin D. The research work also discussed about the presence of vitamin D responsive elements in the promoter region of IL-10 R gene and the possible use of agents modulating the receptor for IL-10 in inflammatory skin conditions [12]. In a study conducted by Bakdash G et al., isolated monocytes derived DCs were treated with calcitriol, calcidiol, and cholecalciferol in the culture medium. Both, calcitriol and calcidiol inhibited the DC maturation, decreased the production of Tumor necrosis factor alpha (TNF-α) and increased IL-10 production [13]. Vitamin D Response Elements (VDRE) have been located in the genes coding for IL-10 cytokine along with IL-2, IL-12B, which could explain the transcriptional regulation of these cytokines by 1, 25-dihydroxy vitamin D3 [14]. With this background, the study was undertaken to study the influence of vitamin D status and IL-10 gene promoter polymorphism (rs1800871) on circulating IL-10 cytokine levels in apparently healthy South Indian population.

MATERIALS AND METHODS

The present cross-sectional study was done in the Department of Clinical Pharmacology, JIPMER, Puducherry, India, from January 2016 to December 2017. The study was approved by Institute Ethics Committee for human studies (Reg.No. ECR/342/Inst/PY/2013, Approval no: JIP/IEC/2015/20/725 dated 21/12/2015).

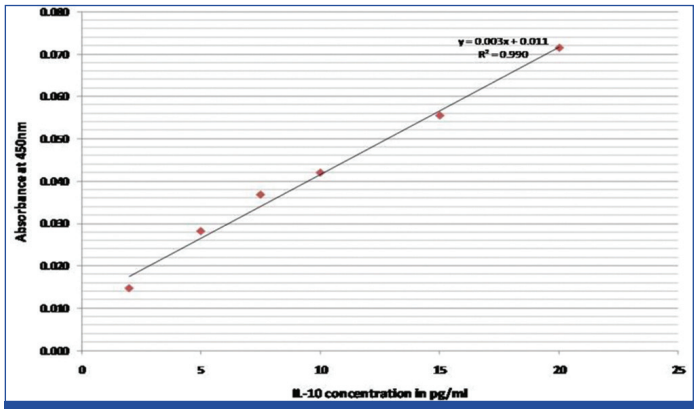
Inclusion criteria: A total of 101 unrelated apparently healthy volunteers of either sex, age more than 18 years, residing in Tamil Nadu or Puducherry, and speaking one of the south Indian languages as mother tongue for more than three generations, were recruited for the study, after obtaining written informed consent. The volunteers were screened and the individuals without any health complaints, and not suffering from any disease conditions were considered as apparently healthy [15].

Exclusion criteria: During screening individuals with any health complaints, individuals suffering from any disease conditions, and not giving consent were excluded.

Study Procedure

Serum Vitamin D level, plasma IL-10 level, and frequency of IL-10 rs1800871 polymorphism were established in them. The volunteers found to be vitamin D deficient were supplemented with vitamin D 60,000 IU per week for 8 weeks (2 months). 6 mL of venous blood was collected from each volunteer. 3 mL of blood was taken in serum tube for separation; serum was stored at -80°C till analysis. The other 3 mL of blood was collected in polypropylene tubes containing 10% Ethylenediaminetetraacetic Acid (EDTA). The samples were centrifuged at 2500 rpm for 10 minutes at 4°C. The plasma was separated and stored at -80°C till IL-10 analysis. The cellular fraction was also stored and used for genetic analysis.

Serum 25-hydroxy vitamin D {25-(OH)D} levels were measured using chemiluminescence assay as per the manufacturer's instructions (ADVIA Centaur Vitamin D total assay, Siemens healthcare diagnostics, USA). Both, total 25-OH vitamin D2 and D3 were measured in the assay. The lower limit of quantification was 4.2 ng/mL and detection range was up to 150 ng/mL. The plasma IL-10 cytokine levels were measured using sandwich ELISA according to manufacturer's (RayBio, Norcross, Georgia) instructions. The concentrations of IL-10 in plasma samples were calculated after plotting graph of standard concentration vs absorbance at 450 nm [Table/Fig-1].



[Table/Fig-1]: Standard curve IL-10.

DNA extraction was done by the standard phenol-chloroform method [16]. The stored cellular fraction of samples was added with Red Blood Cells (RBC) lysis solution which causes swelling and subsequent rupture of RBCs. Once the RBCs were removed, the nucleated pellet was lysed by adding White Blood Cells (WBC) lysis solution to disrupt the WBC membrane, and subsequent addition of 10% Sodium Dodecyl Sulphate (SDS) to remove lipids in the cell membrane. In the next step proteinase K was added to digest proteins including DNases and RNases which will free the Deoxyribonucleic Acid (DNA) from the chromatin. After successful disruption of WBC membrane, chloroform was added for the inactivation of remaining proteins and to remove the excess proteinase K. Equilibrated phenol was added to the samples to help the DNA to remain in aqueous phase. Subsequently, chloroform and octanol were added to remove any traces of phenol from the nucleic acid preparation. Finally, addition of ice cold absolute ethanol precipitated high molecular weight DNA. Precipitated DNA samples were adequately washed with 70% ethanol, dried and dissolved in Tris EDTA buffer for dissolution at 37°C overnight. After complete dissolution, the DNA samples were stored at 4°C for further use. Extracted DNA was quantified by NanoDropTM and genotyping was performed by RT-PCR. TaqMan assay probe for rs1800871 (assay id: C___1747362_10; catalogue number: 4351379) was used in RT-PCR to detect genetic variation. ABI prism 7300 Real-time PCR system supplied by Applied Biosystems (ABI Prism 7300, Foster City, CA, USA) was used.

STATISTICAL ANALYSIS

The data were assessed for normality using Kolmogrov-Smirnov test. Values were expressed as median, IQR. Proportions were described as number with percentage. Genotype and allele frequencies were compared with 1000 genome population and studies done in Indian population by Chi-square test. Kruskal-Wallis test was used to analyse IL-10 levels across different genotype groups, and vitamin D level categories. Spearman's correlation was done to assess the association of vitamin D with other parameters such as age, Body Mass Index (BMI), sunlight exposure, and IL-10 level of the population. The p-value≤0.05 was considered as statistically significant. Statistical analyses were done using Statistical Package for Social Sciences (SPSS), International Business Machines (IBM) version 19.0, and GraphPadInstat version 3.0.

RESULTS

The baseline demographic characteristics of study participants were shown in [Table/Fig-2]. The median age of the participants was 32 years; among them 43.6% were males. The median serum vitamin D level of the study volunteers was found to be 18.21 ng/mL with IQR between 11.3 and, 23.08 ng/mL. Both, median and mean values of serum vitamin D level were provided to allow for comparison with other published studies.

Sl. No.	Characteristics	Apparently healthy population (N=101)
1	Age (years)	32 (28, 43)
2	Gender (male, %)	44 (43.6%)
3	Weight (kg)	64 (55, 71.5)
4	BMI (kg/m^2)	24.49 (21.73, 26.74)
5	Sun exposure (in hours)	2 (0.63, 4.0)
9	IL-10 (pg/mL)	9.04 (7.75, 11.34)
10	Vitamin D (ng/mL) median (IQR)	18.21 (11.3, 23.08)
	Mean (SD)	17.88 (7.18)
11	Vitamin D deficiency, n (%)	60 (59.4%)

[Table/Fig-2]: Baseline demographic characteristics of study participants.
Values expressed as the median (interquartile range), and gender expressed as number of male (%).
BMI: Body mass index; IL-10: Interleukin 10

The frequency of CC, CT, TT genotypes in the study population was 28.7%, 43.6%, and 27.7%, respectively. The frequency of C and T alleles were 50.5% and 49.5%, respectively [Table/Fig-3,4]. The genotype distribution of IL-10 rs1800871 polymorphism did not deviate from the Hardy-Weinberg equilibrium.

Genotype/ Allele IL-10, rs1800871, -819 C>T	Present study (n=101)	AFR (n=661)	AMR* (n=347)	EAS* (n=504)	EUR* (n=503)	SAS (n=489)
CC	29 (28.7)	217 (32.8)	160 (46.1)	50 (10)	291 (57.8)	149 (30.5)
CT	44 (43.6)	312 (47.2)	143 (41.2)	227 (45)	183 (36.4)	232 (47.40
TT	28 (27.7)	132 (20)	44 (12.7)	227 (45)	29 (5.8)	108 (22.1)
C	102 (50.5)	746 (56.4)	463 (66.7)	327 (32.4)	765 (76)	530 (54.2)
T	100 (49.5)	576 (43.6)	231 (33.3)	681 (67.6)	241 (24)	448 (45.8)

[Table/Fig-3]: Comparison of genetic frequency of rs1800871 with the 1000 Genomes project data [6,24].
Values expressed as n(%); *p<0.05 (Chi-square test)
AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian

The median plasma IL-10 levels were 9.69 pg/mL, 9.83 pg/mL and 8.35 pg/mL for the rs1800871 CC, CT and TT genotypes, respectively. Plasma IL-10 levels were not significantly different across genotypes (p-value=0.091), even though the median level of homozygous mutant (TT) genotype was observed to be less [Table/Fig-5]. Based on the serum vitamin D levels, the study population was divided in to four groups with vitamin D levels of <10 ng/mL, 10-20 ng/mL, 20-30 ng/mL and >30 ng/mL. The median IL-10 levels were 8.92 pg/mL, 8.48pg/mL, 9.32 pg/mL, and 11.15 pg/mL, respectively in the vitamin D subgroups [Table/Fig-6].

The correlation between serum vitamin D level and other parameters such as age, BMI, sunlight exposure, plasma IL-10 levels was analysed. Serum vitamin level was found to be positively correlated with age (correlation coefficient 0.220, p-value=0.027) and sunlight exposure (correlation coefficient 0.447, p-value <0.0001). Vitamin D level was found to be negatively correlated with BMI (correlation coefficient -0.226, p-value=0.023). No correlation was observed between vitamin D and IL-10 levels (correlation coefficient 0.147, p-value=0.143) [Table/Fig-7].

Genotype/ Allele rs1800871	Present study (n=101)	Madeshiya AK et al., 2017 [25]* (n=386)	Khatri R et al., 2011 [22] (n=60)	Pooja S et al., 2012 [26] (n=200)	Raeisza-deh Jahromi S et al., 2015 [27] (n=393)	Indhu-mathi S et al., 2017 [28]* (n=360)
CC	29 (28.7)	171 (44.3)	20 (33.3)	57 (28.5)	126 (32.1)	28 (7.8)
CT	44 (43.6)	166 (43.0)	33 (55)	78 (39.0)	178 (45.3)	222 (61.7)
TT	28 (27.7)	49 (12.7)	7 (11.7)	65 (32.5)	89 (22.6)	110 (30.5)
C	102 (50.5)	508 (65.8)	73 (60.8)	192 (48)	430 (54.7)	329 (45.7)
T	100 (49.5)	264 (34.2)	47 (39.2)	208 (52)	356 (45.3)	391 (54.3)

[Table/Fig-4]: Comparison of genetic frequency of SNP rs1800871 with other reported studies conducted in Indian population [22,25-28].
Values expressed as n(%); *p<0.05 (Chi-square test)

IL-10 rs1800871 Genotype	IL-10 level (pg/mL)
CC	9.69 (7.54, 12.38)
CT	9.83 (7.93, 11.98)
TT	8.35 (7.13, 9.33)

[Table/Fig-5]: IL-10 cytokine level among the different genotypes.
Values expressed as the median (IQR).
p-value=0.091 (Kruskal-wallis test, not significant across genotypes)

Vitamin D level	IL-10 level (pg/mL)
Less than 10 ng/mL, n=16	8.92 (7.13, 11.02)
10-20 ng/mL, n=44	8.48 (9.45, 10.27)
20-30 ng/mL, n=37	9.32 (8.34, 11.45)
More than 30 ng/mL, n=4	11.15 (7.86, 14.31)

[Table/Fig-6]: Vitamin D status and IL-10 level in healthy people (n=101).
Values expressed as the median (IQR)
p-value=0.40 (Kruskal-wallis test, comparison of medians across the four unmatched groups)

Factor	Healthy volunteers (n=101)	
	Correlation coefficient	p-value
Age (years)	0.220	0.027
BMI (kg/m^2)	-0.226	0.023
Sunlight exposure (in hr)	0.447	<0.0001
IL-10 (pg/mL)	0.147	0.143

[Table/Fig-7]: Correlation between vitamin D level and other parameters in the study population.
Non parametric Spearman correlation test was applied; BMI: Body Mass Index

DISCUSSION

The IL-10 is a major anti-inflammatory cytokine, which plays a vital role in regulating the immune response and maintaining immune balance. Genetic polymorphisms involving the promoter region of the IL-10 gene can potentially alter the expression of IL-10. Vitamin D, the sunshine hormone has shown a significant contribution in immune response, potentially through its influence over the IL-10 cytokine. According to Endocrine society guidelines, vitamin D level <20 ng/mL is considered as deficient [17]. In the present study, 59.4% of the individuals had serum vitamin D level in the deficient range. The mean vitamin D level seen among the study participants was 17.88 (SD, 7.18). Ritu G and Gupta A have compiled the studies reporting vitamin D status in healthy Indian population and reported that the mean vitamin D level ranges from 3.19 to 52.9 ng/mL. They also reported the frequency of deficiency as 70 to 100% [18]. A recent meta-analysis done by Selvarajan S *et al*., which included 40 studies from India, has pointed out the mean vitamin D level as 14.16 ng/mL (95% CI, 13.27-15.05 ng/mL) among healthy population. The mean vitamin D level of the population from south zone was found to be 17.45 ng/mL (95% CI, 15.74-19.16 ng/mL) [19]. Mean vitamin D level noted in present study, which included volunteers from Tamil

Nadu and Puducherry, was within this reported range mentioned above. The mean or median values of IL-10 cytokine level in the peripheral circulation of healthy individuals were reported to be between 7.86 and 16.11 pg/mL [20-22]. A study conducted to find out the levels of various cytokines in different age group of individuals, observed a median IL-10 level of 12.6 pg/mL (IQR, 8.5-16.7 pg/mL) [21]. The median value observed in the present study was 9.04 (IQR, 7.75-11.34) pg/mL, which is within the range reported in the scientific literature.

The measured IL-10 levels were compared across different vitamin D status categories. Individuals with serum vitamin D level in sufficient range (>30 ng/mL) had median IL-10 level of 11.15 (IQR, 7.86-14.31) pg/mL, compared to the deficient, and insufficient categories. But, the comparison of IL-10 levels among different vitamin D status groups did not meet statistical significance. Most of the individuals in the deficient range (60 out of 101 volunteers), and having only 4% of the volunteers with sufficient vitamin D level could be the reasons for the insignificance. Increased expression of IL-10 was demonstrated in malignant conditions, like, melanoma, squamous, basal cell carcinoma, and lymphomas. Raised IL-10 levels were evaluated to be a poor prognostic marker in malignant lymphomas. Correlation with increased expression of IL-10 was found with autoimmune diseases, such as SLE, bullous pemphigoid and systemic sclerosis. Decreased expression or reduced IL-10 production was seen with inflammatory diseases like rheumatoid arthritis, psoriasis, inflammatory bowel disease, multiple sclerosis, and allergic contact dermatitis, where elevated type 1 response cytokines were involved in pathogenesis. In allergic disorders like atopic dermatitis and asthma, over-expression of IL-10 has been considered as a counter-regulatory response to limit inflammation. Based on these findings, IL-10 was evaluated as a therapeutic target and recombinant IL-10 therapy has been tried in rheumatoid arthritis, crohn's disease, and Human Immunodeficiency Virus (HIV) infection with unsatisfactory results. IL-10 therapy in psoriasis vulgaris and associated arthritis was found to be beneficial with good to moderate response [1,23].

The frequency of IL-10 gene promoter polymorphism (rs1800871, -819 C>T) was established in healthy volunteers. The SNP is reported to be in linkage disequilibrium with rs1800872, another SNP in IL-10 gene promoter region [5]. The genotype frequency of rs1800871 in the present study was compared to 1000 genome population and it was found that, the frequency was similar to African and South Asian population [Table/Fig-3] [6,24]. The South Asian population studied in 1000 genome project included Bengali people from Bangladesh, Gujarati Indians in Houston, Indian Telugu from United Kingdom (UK), Punjabi people from Lahore, and Sri Lankan Tamil from the UK. The authors also compared the genotype frequency observed with other reported studies conducted in Indian population [Table/Fig-4]. Three studies were conducted in North Indian population and two studies from South Indian population were included for comparison [22,25,26]. Among South Indian studies, Raeiszadeh Jahromi S et al., had included people from Karnataka, and Indhumathi S et al., had included South Indian Tamilian population from Puducherry and Tamil Nadu [27,28]. The allele frequency observed in the present study was consistent with the frequencies reported in south Indian population. The promoter region polymorphism of IL-10 had known to affect IL-10 protein production [29]. The authors compared the plasma IL-10 levels among wild, heterozygous mutant, and homozygous mutant genotypes, but no statistical difference could be observed. The individuals carrying homozygous mutation had lower plasma IL-10 levels compared to persons carrying normal allele, but the difference was not found to be statistically significant.

Age and exposure to sunlight were positively correlated with serum vitamin D levels and showed statistical significance in the population studied. BMI was negatively correlated with vitamin D level. The inverse relation between BMI and vitamin D can be explained by sequestration of vitamin D metabolites as well as the slow release of them from excess body fat seen in people with high BMI [30]. Vitamin D receptors are distributed and expressed in various cells of immune system including T lymphocytes, B lymphocytes, monocytes, DCs, natural killer cells and haematopoietic cells in bone marrow. In addition to expressing vitamin D receptor (VDR), some of the immune cells including lymphocytes and DCs also do express 1-α hydroxylase which is involved in local production of active vitamin D. This active vitamin D also acts in paracrine and autocrine fashion to exert its effects on immune system [31]. Treatment with vitamin D has led to significant improvement in IL-10 levels. Ashtari F et al., did a randomised controlled trial in patients with multiple sclerosis. In the study, administration of 50,000 IU of vitamin D every 5 days for 90 days increased the serum IL-10 level from 12.58±11.97 pg/mL to 13.76±18.95 pg/mL. The increase was not statistically significant, but high variation in the serum IL-10 level was noted. After adjusting for confounding factors such as age, gender, and disease severity the positive correlation between vitamin D and IL-10 was reported [11]. A meta-analysis done on randomised controlled studies of vitamin D supplementation on inflammatory markers, suggest low serum vitamin D level at baseline, higher increase in vitamin D level after intervention, and profound inflammatory conditions were the factors determining outcomes of such trials [32]. Unable to appreciate the relation between vitamin D and IL-10 level in healthy population of the present study could be because the immune system is at balance among them which was disturbed in individuals with inflammatory disorders. Hence, the difference in IL-10 levels according to vitamin D status might get exposed during infection or altered immunology as in the case of immunological disorders.

Limitation(s)

The authors measured only the circulating levels of IL-10. Studying the whole panel of inflammatory and anti-inflammatory markers will provide broader information rather than studying only IL-10 cytokine, and analysing the IL-10 expression at the cellular level would have been more appropriate than measuring peripheral circulating levels of the cytokine. rs1800871 was the only polymorphism studied in the present study. Studying all the significant SNPs reported, would provide clear picture all the influence of genetic polymorphisms over the IL-10 phenotype.

Future Perspective

The authors have reported genotype frequency, IL10 levels, and vitamin D status among healthy population. This could help the future researchers to have data to compare their findings. The observed genotype and allele frequency was compared to populations of 1000 genome project and other Indian studies to express the consistency of the present study's observation with south Indian population. The differences in circulating IL10 levels among different vitamin D status groups, and genotype groups based on rs1800871 were not statistically significant. The present study might not be adequately powered to distinguish these differences. Conducting similar studies with larger number sample size could help to find the answers.

The cutoff levels of vitamin D status definition were based on the vitamin's role in bone-mineral homeostasis. Since, the optimal levels for the normal immune function were not established in the literature [33], the authors followed the vitamin D status classification suggested for bone function. Vitamin D has pleiotropic functions other than bone health, hence defining the cut-off level of adequacy for its multiple actions, is important to be looked for in future research. It has been insisted that, the extra-skeletal, non-classical actions of vitamin D are mediated in autocrine, as well as, paracrine manner in addition to the endocrine mechanism [34]. These additional mechanisms would complicate the process of finding the optimal level for each pleiotropic action.

CONCLUSION(S)

No significant influence of vitamin D status and rs1800871 polymorphism on IL-10 level was observed in the study population. But the observed data suggest that, both the vitamin D status and rs1800871 polymorphism could play a contributory role in determining peripheral circulating IL-10 cytokine levels, which requires further in-depth analysis for scientific evidence.

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