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ORIGINAL RESEARCH

Prevalence of C/T Allele of Il1b Gene Along with its Relationship in Polymorphism (-511C/T) with Vitiligo

Dr Rajeshwari[1], Dr Shruti Math[2], Dr Amin Syed Moinuddin[3], Dr Chaitanya Namdeo[4], Dr Rini Sharma[5]

[1]Assistant Professor, Department of Dermatology, Venereology and Leprosy. Faculty of Medical Sciences, Khaja Banda Nawaz University KBNU, Kalaburagi (Karnataka) India.
[2]Assistant Professor, Department of Dermatology, venereology and Leprosy, Ashwini Rural Medical College Hospital and Research Centre, Maharashtra University of Health Sciences (MUHS), Maharashtra, India.
[3]Associate Professor, Department of Dermatology, Venereology and Leprosy, Sri Aurobindo Medical College and Postgraduate Institute, Indore. Sri Aurobindo University Indore (M.P) India.
[4]Professor, Department of Dermatology, Venereology and Leprosy. Sri Aurobindo medical college and postgraduate Institute Indore. Sri Aurobindo University Indore (M.P) India.
[5]Department of Dermatology, venereology and Leprosy, Sri Aurobindo medical college and Postgraduate Institute Indore, Sri Aurobindo University Indore (M.P), India.

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ABSTRACT

Background: Vitiligo is a condition where white patches appear on the skin due to lack of melanin pigment. It can appear on any part of the body, but most commonly affected areas are face, neck, and hand. It can cause significant psychological and social distress in patient suffering from it. Methodology- The study was conducted at Central Research Laboratory and Department of Skin and VD, Sri Aurobindo Medical College and Post Graduate Institute, Indore, from Oct 2014 to Jun 2016. 84 known patients of vitiligo were recruited for the study. Patients were diagnosed to have vitiligo based on history, duration, progression of disease presence of depigmented patch and appearance of chalky white on wood's lamp examination. DNA was extracted from EDTA blood using Himedia HiPurA kit. The IL1B -511C/T SNP was genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method. Results- C allele was observed in 53% of cases and 50.6% of controls whereas T allele was present in 47 % of cases and 53% of controls. No association of T allele with the presence of vitiligo was seen in present study (p = 0.910) Conclusion- No significant association of IL1B -511C/T (rs 16944) polymorphism at genotype level and allele level was seen with the vitiligo in this study. None of the model was found associated with vitiligo**.**

Keywords- Vitiligo, IL1B, polymorphism, white patches, genotype

Corresponding Author: Dr. Rajeshwari, Assistant professor Department of Dermatology, Venereology and Leprosy. Faculty of Medical Sciences, Khaja Banda Nawaz University KBNU, Kalaburagi (Karnataka) India.
Email: rajumnaik5@gmail.com

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INTRODUCTION

Vitiligo is a common skin disorder that affects between 0.1-2% of the world's population.[1-2] Vitiligo is an acquired hypomelanotic disorder characterized by circumscribed depigmented macules in the skin resulting from the loss of functional melanocytes from the cutaneous epidermis. Although not associated with other symptoms it can cause significant psychological and social distress.[3-7] The disorder results in substantial cosmetic disfigurement, particularly in dark skinned persons and even limited vitiligo in such an individual is socially detrimental.

The most accepted view in vitiligo pathogenesis is the interaction between genetic and non-genetic factors that influence melanocyte survival and function [8-9]. Neurochemical mediators secreted by the nerve endings such as acetylcholine and catecholamines might lead to the destruction of melanocytes.[10] Vitiligo is frequently associated with positive family history.[11-12] The inheritance pattern of vitiligo does not follow the simple Mendelian pattern and its mode of heredity suggests that it is a polygenic disease. Vitiligo seems to be a complex hereditary disease governed by a set of recessive alleles situated at several unlinked autosomal loci which may be involved in the generation of oxidative stress, melanin synthesis, autoimmunity etc. that could collectively confer the vitiligo phenotype.[13-14]

Interleukin-1 (IL-1) is the pro-inflammatory cytokine that have proved essential at the onset of inflammation and during an innate immune response.[15-16] It is a mediator of the acute phase of inflammation by induction of local and systemic responses.[17] Amongst others IL-1 induces the expression of adhesion molecules on endothelial cells, which are required for the infiltration of the stressed tissue by inflammatory and immunocompetent cells. It also induces pain sensitivity, fever, vasodilation and hypotension.[18] IL-1 is strongly expressed by monocytes, tissue macrophages and dendritic cells, but is also produced by B lymphocytes, NK cells and epithelial cells.[19] Of these 2 IL-1 forms (Il-1α and Il-1β), IL-1β is more frequently involved in chronic inflammatory disease and is subject to intricate pathways of control at many different levels, which limits the production of IL-1β and thereby prevents inadvertent activation of inflammation.

Keeping the thought hereditary pattern of vitiligo and autoimmunity as one of the pathogenesis for vitiligo, current study was designed to see the prevalence of C &T allele of IL1B gene along with its relationship in polymorphism(-511C/T) with vitiligo.

MATERIALS & METHODS

This prospective case control study was conducted at Central Research Laboratory and Department of Skin and VD, Sri Aurobindo Medical College and Post Graduate Institute, Indore. The duration of the study was from Oct 2014 to Jun 2016.The sample size taken for the study was 84 patients. Written informed written consent was taken from all the patients. Patients belonging to both genders, age group and all types of vitiligo were included in the study. Patients with other depigmentary disorders like; Albinism, Lichen sclerosus et atrophicus, Lupus erythematosus, Nevus depigmentosis, Nevus anaemicus, Halo nevus, Leprosy, Incontenenti pigmenti, leucoderma, Post-inflammatory hypopigmentation, Idiopathic guttate hypomelanosis, pityriasis versicolor were excluded from the study. Patients were diagnosed to have vitiligo based on history, duration, progression of disease presence of depigmented patch and appearance of chalky white on wood's lamp examination. Seventy- six healthy controls were also recruited for comparative analysis. Detailed clinical examination was done on all patients to rule out other systemic disorders. History from all the patients was taken regarding duration of present illness, past history of vitiligo and other systemic disorders, past medical history, family history. General Examination like pallor, icterus, clubbing, oedema, and lymphadenopathy were checked and were checked and recorded in all patients. 5ml of blood samples were collected in plain and EDTA tube from peripheral veins under

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aseptic precautions. Serum was separated by centrifugation of plain tube at 2000rpm and serum was tested for presence of TSH and diabetes mellitus.

DNA Isolation

DNA was extracted from EDTA blood using Himedia HiPurA kit.

200 µl of Lysis Solution was added to 200 µl of whole blood. 20 µl of the reconstituted Proteinase K solution (20 mg/ml) was added and was incubated at 55°C for 10 minutes.

200 µl of ethanol (96-100%) was added to the lysate obtained from the above step for preparation of lysate for binding to the spin column. Lysate obtained from step 4 was transfered into the spin column provided. Column was centrifuged at 10,000 rpm for 1 minute. Discarded the flow-through liquid and place the column in a new 2.0 ml collection tube. 500 µl of diluted Prewash Solution was added to the column and centrifuge at 10,000 rpm) for 1 minute. Discarded the flow-through liquid and re-use the same collection tube with the column. 500 µl of diluted Wash Solution was added to the column and centrifuge at 13000rpm. Discarded the collection tube containing the flow through liquid and place the column in a new 2.0 ml collection tube. 100 µl of the Elution Buffer (ET) was directly added onto the column without spilling to the sides. Incubated for 1 minute at room temperature (15-25°C) and centrifuged at 10,000 rpm for 1 minute to elute the DNA. The eluated sample was transferred to a fresh capped 2ml collection tube for longer DNA storage.

Genotyping of IL1B promoter polymorphism: The IL1B -511C/T SNP was genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method. The PCR mix consist of the 0.2µg of DNA, 10pM of the both forward primer (5'-GTTTAGGAATCTTCCCACTT-3') and reverse primer(5'-TGGCATTGATCTGGTTCATC-3') along with PCR Assay Buffer (100mM Tris (pH 8.3), 15mM $MgCl_2$, 500mM KCl), 200µM of dNTP's and 1U of *Taq* polymerase. The PCR profile was as follows: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturing at 95°C for 60 s, annealing at 50°C for 60 s and extension at 72°C for 60 s and a final incubation at 72°C for 5 min. The resulting amplified PCR product (305bp) was digested with 10 unit of Bsu36I (New England Biolabs Inc., UK) restriction enzyme and incubated at 37°C for 12 hours. After digestion the product were run on 2% agarose gel and after staining with ethidium bromide; visualized on trans illuminator. Restriction enzyme Bsu36I cuts the IL1B amplicon at the ancestral allele 'T' to give 113 bp and 192 bp products but the polymorphic C allele remains uncut. Data was entered in Microsoft excel and analysed on Graph Pad Software (demo version).

RESULTS

TABLE 1: IL 1b Allele Frequency in Case and Control

	Case	**Control**	**P-value, OR (95%CI)**
C	89(53)	74(50.6)	0.9100, 0.969 (0.613- 1.491)
T	81(47)	72(49.4)	

TABLE 2: Dominant and Recessive model

	Case	**Control**	**P -Value**
Dominant Model			
CC+CT	64	53	0.7139
TT	20	20	
Recessive Model			
CC	23	21	0.8605
CT+TT	61	52	

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TABLE 3: Gender wise genotype frequency in cases

	Male	Female	P-value
CC	13 (28.9)	10 (25.6)	**0.9125**
CT	22 (48.9)	19 (48.7)	
TT	10 (22.2)	10 (25.6)	

TABLE 4: Gender wise Genotype frequency in controls

	Male	Female	P-value
CC	16 (33.3)	5(20.0)	**0.4495**
CT	19 (39.6)	13(52.0)	
TT	13 (27.1)	7(28.0)	

Table 3 and 4 shows the genotype frequency in according to gender in cases and controls.

DISCUSSION

In our study**,** C allele was observed in 53% of cases and 50.6% of controls whereas T allele was present in 47 % of cases and 53% of controls. No association of T allele with the presence of vitiligo was seen in present study (p = 0.910).

CT genotype was pooled with CC and TT genotype to see the dominant and recessive model association with vitiligo. None of the model was found associated with vitiligo.

The genotype frequency was similar in males and females in both cases and controls.

No significant association of Il1b -511C/T (rs 16944) polymorphism at genotype level and allele level was seen with the vitiligo in our study. However, in Contrast to our study Laddha et al[20] reported the positive association of Il1b -511C/T (rs 16944) polymorphism with both generalised and localised vitiligo. Both genotype and allelic frequencies for IL1b promoter polymorphism differed significantly between AV patients and controls ($p<0.0001$ and $p<0.0001$ respectively). However, the genotype as well as allelic frequencies for SV patients did not differ as compared to controls ($p = 0.08$ and $p = 0.06$ respectively).

Tarlé et al[21] conducted similar type of study and analysed re-association of various SNPs of IL-1B gene [rs1143634, rs1143633 and rs3136558] in vitiligo patients. They also did not observe any significant association of any SNPs in any vitiligo patients.

The discripency in results may be due to different type of vitiligo patients (stable vs active and localised vs generalised) selected in different studies.

CONCLUSION

No significant association of Il1b -511C/T (rs 16944) polymorphism at genotype level and allele level was seen with the vitiligo in this study. CT genotype was pooled with CC and TT genotype to see the dominant and recessive model association with vitiligo. None of the model was found associated with vitiligo.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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ISSN: 0975-3583,0976-2833 VOL14, ISSUE 02, 2023

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