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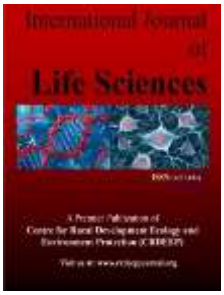

Full Length Research Paper

Genetic polymorphism of CD14 Promoter (rs2569190) gene and its association with *Plasmodium falciparum* Malaria in Vindhyan Population

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ABSTRACT

CD14 is a multifunctional receptor expressed on many cell types and has been shown to mediate immune response resulting in the activation of an inflammatory cascade, with polymorphism of its promoter (rs2569190) found to be associated with susceptibility to several diseases. In malaria infection, the CD14 gene demonstrated a pathogenic profile in regulating experimental cerebral malaria, with reports of elevated levels of soluble CD14 in serum of patients but no definitive conclusion. We present a detailed analysis of genetic diversity of CD14 promoter gene (snp –159 C/T; rs2519190) polymorphism between a malaria-infected group and uninfected controls and its association with clinical parameters of disease. Since host genetic factors are estimated to account for one quarter of the total variability inmalaria infection and severity, the possibility that a genetic variant acting as a cis-regulatory factor of CD14 expression is also explored. PCR amplification with specific primers gave 295bp product which was digested with BsuR1 enzyme for 16 h at 37˚C. The wild-type genotype (CC) was digested at restriction site. The wild type C allele having restriction site for HaeIII enzyme, after mutation C allele concerted in to Mutant T allele where restriction site was disappeared. Case (Malarial patient) – Control (Healthy population) study in respect of Chi Square Test reveals that two type allele present in three genotype CC, TC, CC. Statistical analysis, between Malarial patient and healthy control reveals genotypic association as χ^2 (P Value) = 6.697, (0.0351). Allele frequency is also significantly associated as χ^2 (P Value) = 6.048, (0.0139*) whereas statistical data of carriage rate suggest no association between case and control. Our findings suggest weak or possible association of CD14 gene polymorphism with malaria.*

Introduction

Inflammatory reactions, secondary to pathogenic stimuli, are important for the initiation of necessary immune response for combating the external stimuli when effectively and efficiently deployed. Infection with *Plasmodium falciparum*, the causative agent of malaria is one such pathogen for which the inflammatory cascade would be necessary, considering possible infection severity, especially in non immune travelers or children [1-3]. Several innate immunity genes have been implicated, at one time or another, as having significant roles in disease severity, infection outcome, or development of possible sequelae, post infection. One such gene is CD14, a co receptor to the toll-like receptor (TLR)-4/MD2 complex on leukocytes, and expressed on many cell types. It has been demonstrated to mediate innate immune response due to its specificity for bacterial lipopolysaccharide and consequent pro-inflammatory signaling and initiation of phagocytosis. It lends its capacity to T-cell plasticity, driving a TH1 differentiation, especially with infections such as malaria [4-7].

In published reports, genetic variations leading to dysfunctional monocyte functions have been implicated in disease susceptibility and outcome, probably clarifying how human populations have adapted to major infectious diseases through the ages. Genetic variants of the CD14 gene (a C-to-T transition, snp −159 C/T) have been shown to be associated with susceptibility to multiple diseases and conditions including allergic rhinitis,15 functional dyspepsia,16 inflammatory bowel disease, gram-negative bacteria, and respiratory infections, including brucellosis. Its significance in allergic reactions and outcome in patients with asthma have also been documented. In human, malaria infections particularly, elevated levels of soluble CD14 have been found in patients with acute infections, concluding that soluble CD14 levels may play a critical role in disease severity [8,5]. A murine infection model has been the clearest

to delineate a specific pathogenic role for CD14 during experimental cerebral malaria infection, showing a protective outcome in *Plasmodium berghei*–infected transgenic CD14 mice. Although differing outcomes have been reported for the critical role of TLRs alongside CD14, its c receptor, in mediating disease susceptibility or outcome in malaria infection, it is abundantly clear that this promoter gene potentially has a significant role in disease outcome [9-12].

Our objective was to evaluate CD 14 gene polymorphism pattern in our population and its association with malaria

Materials and Methods

Study Population

The study population consisted of 390 unrelated subjects comprising of 180 *plasmodium falciperum* infected patients and 210 ethnically matched controls of Vindhyan population (Central India) were included in this study. Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi.

Anthropometric Measurements

Height and Weight were measured in light clothes and without shoes in standing position as per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences..

Life Style Factor

We selected some life style habit such as smoking and drinking in our population. Questionnaire based life style data from both malarial patient and healthy population reveals susceptibility to *plasmodium falciparum* malaria were analysed by statistical tool, Graph pad Prism 3.3 software.

Blood Collection and Plasma/Serum Separation

Blood samples were obtained from the malarial patient and healthy population in veils with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective veils were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C.

Genomic DNA Isolation and Quantification

DNA isolation method from human blood; Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was then allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl. of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) were added to remove most of the non-nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization. Agarose Gel Electrophoresis: Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used. In brief, 4-5 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solution (0.5 µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA). Lambda DNA *Eco*RI / *Hind* III double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker after completion of electrophoresis, the DNA bands were visualized and photographed using an UV transilluminator (312 nm) and gel documentation system (Vilber Lourmate, Cedex 1, France) respectively.

Quantification of isolated DNA: The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using nano drop (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. Gel electrophoresis of the genomic DNAs was carried out

for qualitative estimation of extracted DNA. A good DNA appears as bright and sharp band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used.

Polymorphism screening
In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest. All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative control was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative control used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence. Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different from the normal recognition sequence. Such aberrant cutting is called "star activity" which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µg of DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO). The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

Detection of CD14 Promoter gene (rs2569190)
PCR amplification with specific primers gave 295bp product which was digested with *BsuR1* enzyme for 16 h at 37˚C. The wild-type genotype (CC) was digested at restriction site. The wild type C allele having restriction site for *Hae*III enzyme, after mutation C allele concerted in to Mutant T allele where restriction site was disappeared.

Primer Sequences
The oligonucleotides sequences (primers) were designed for SNP (C-159T), rs2569190. This primer were early used in previous study.
CD14 promoter (C-159T); forward primer – 5´-ATCATCCTTTTCCCACACC-3´
CD14 promoter (C-159T); reverse primer - 5´-AACTCTTCGGCTGCCTCT-3´

PCR Reaction Mixture
We amplified each DNA sample as 25 µl of PCR reaction mixture containing 5 µl template DNA (final concentration 100-200 ng/ µl), 2.5 µl of 10X Taq polymerase buffer (10 mM TrisHCl pH 8.8, 50 mMKCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; (Merck), 1 µl of 10 mM dNTPs (BangloreGenei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers for CR1 (rs11118133) gene, 0.2 µl of 5U/ µl of Taq DNA polymerase (final concentration 1U; Genetix Biotech Asia Pvt. Ltd.,India) and sterile water to set up the volume of reaction mixture to 25 µl.

Thermal Profile
Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at 94˚C for 2 min and 35 cycles of denaturation at 94˚C for 1 min, annealing at 54˚C for 1 min and extension at 74˚C for 1 min, followed by final extension at 74˚C for 10 min. PCR products were separated on 2% agarose gel (2% w/v, Sigma) using a 100 bp molecular weight (MW) marker.

Restriction Digestion by HaeIII RFLP
Transition from C to T in CD14 promoter gene when amplified by PCR was than incubated with *Hae*III restriction enzyme (New England Biolabs,USA). Digestion of the amplified 295bp PCR product gave two fragments in PAGE of 155bp and 140bp respectively if the product was excisable by *Hae*III. Depending on digestion pattern, individuals were scored as genotype LL when homozygous for presence of *HindII* site, genotype HH when homozygous for absence of *HindII* site and genotype HL in case of heterozygosity (Figure 2).

Results
Anthropometric Measurements
We recruited 180 *P. falciparum* infected malarial patient and 210 healthy population in the respect of anthropometric measurements such as BMI (Body Mass Index) and WHR (Waist Hip Ratio). Comparative study of two parameters BMI and WHR between two groups are not showing any association. BMI factor is not significantly associated with malarial infection in both Women (P=0.2214) and men (P=0.7751) thus WHR is also not showing any association between malarial patient and healthy population in both Women

(P=0.0811) and men (P=0.1106).

Table 1. Comparison of anthropometric parameters of Malaria patients and controls

Characteristics	Cases(180)	Controls(210)	P-value
n(Men/Women)	220(127/53)	240(133/77)	
Age(years)	50.2±10.4	50.1±11.2	0.9277, ns
Height(cm)	160.30±12.3	160.40±11.6	0.9343, ns
Weight (Kg)			
Women	62.6 ±5.3	62.9 ± 5.6	0.5891, ns
Men	63.4±6.8	63.5±6.7	0.8841, ns
BMI (kg/m^2)			
Women	23.4±2.1	23.5 ± 3.2	0.7203, ns
Men	24.5±6.5	24.7± 7.2	0.7751, ns
Waist circumference (cm)			
Women	86.60±7.2	87.45±6.5	0.2214, ns
Men	89.2±6.1	90.1±8.2	0.2260, ns
Hip (cm)			
Women	96.3±5.2	96.5±4.1	0.6715, ns
Men	91.7±3.1	91.6±4.5	0.8017, ns
WHR			
Women	0.93±0.08	0.94±0.02	0.0811, ns
Men	0.97±0.09	0.98±0.01	0.1106, ns

* *denotes level of significant change between case and control.*

Life Style Factor

We collected data of life style factor such as smoking and drinking on both patient and healthy population. We have smoking (P=0.7242) and drinking (P=0.8740) data, suggest no association of these features with susceptibility to *P. falciparum* Malaria.

Table 2. Comparison of Life style factor between Malaria patients and Healthy controls

Life Style Factor	Cases(180)	Controls(210)	P-value
Cigarette smoking (Smoking/ Non Smoking)	68/112	83/127	X^2=0.1245 (p=0.7242), ns
Alcohol consumption (Drinking/ Non- Drinkig)	74/106	88/122	X^2 =0.02514 (P=0.8740), ns

* *denotes level of significant change between case and control.*

Detection of genetic polymorphism in CD 14 Promoter gene (rs2569190)

PCR amplification with specific primers gave 295bp product which was digested with *BsuR1* enzyme for 16 h at 37˚C. The wild-type genotype (CC) was digested at restriction site. The wild type C allele having restriction site for *Hae*III enzyme, after mutation C allele concerted in to Mutant T allele where restriction site was disappeared. The Restriction site containing homozygous genotype (CC) was cut as a doublet of 155bp and 140bp. The heterozygous genotype (TC) was represented as 3 fragments of 295, 155, and 140bp as depicted in figure.

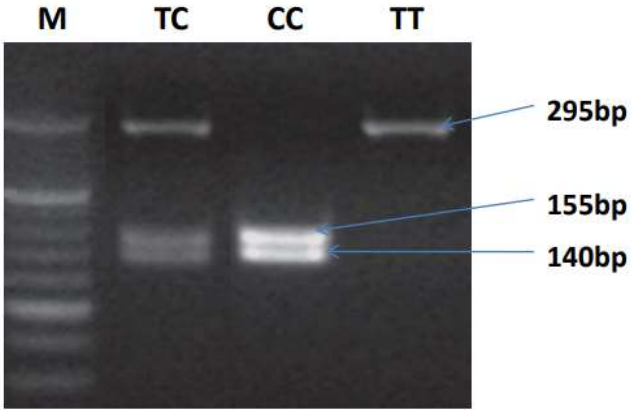


Fig 1: Restriction digestion with BsuR1 enzyme

Statistical analysis depicted in Table No.4.5, containing Genotype frequency, Allele frequency and carriage rate of CD 14 Promoter gene. Case (Malarial patient) – Control (Healthy population) study in respect of Chi Square Test reveals that two type allele present in three genotype CC, TC, CC. Statistical analysis, between Malarial patient and healthy control reveals genotypic association as χ^2 (P

Value) = 6.697, (0.0351*). Allele frequency is also significantly associated as χ^2 (P Value) = 6.048, (0.0139*) whereas statistical data of carriage rate suggest no association between case and control.

Statistical data in the respect of Fisher Exact test between malarial patient and healthy population illustrates P-value and Odds ratio. Statistical analysis reveals TT genotype significantly associated with susceptibility to *P. falciparum* Malaria as P= 0.0340* whereas Odds ratio (0.5050, CI= 0.2686 to 0.9495) suggest protective genotype. Thus allele frequency is also significantly associated with malarial infection (Table no. 4.6). Odds ratio gives information about differences between two allele frequency and genotype frequency in case and control.

Table 2. Frequency distribution and association of Genotype, allele frequency and carriage rate of CD 14 Promoter Gene polymorphism in population of Vindhyan region using Chi Square Test

CD 14 Promoter Gene	CASE (N= 180)		CONTROL (N=210)		CHI SQUARE VALUE X^2(P Value)
	N	%	N	%	
Genotype					
CC	76	42.22	68	32.38	6.697, (0.0351*)
CT	88	48.88	108	51.42	Df=2
TT	16	08.88	34	16.19	
Allele					
C	240	66.66	244	58.09	6.048, (0.0139*)
T	120	33.33	176	41.90	Df=1
Carriage Rate					
C	164	61.19	176	55.34	2.042, (0.1530)
T	104	38.80	142	44.65	Df=1

N – Number of individuals in study group.
%- Genotype allele frequency and carriage rate expressed in percentage.(denotes the level of significant association between case and control)*

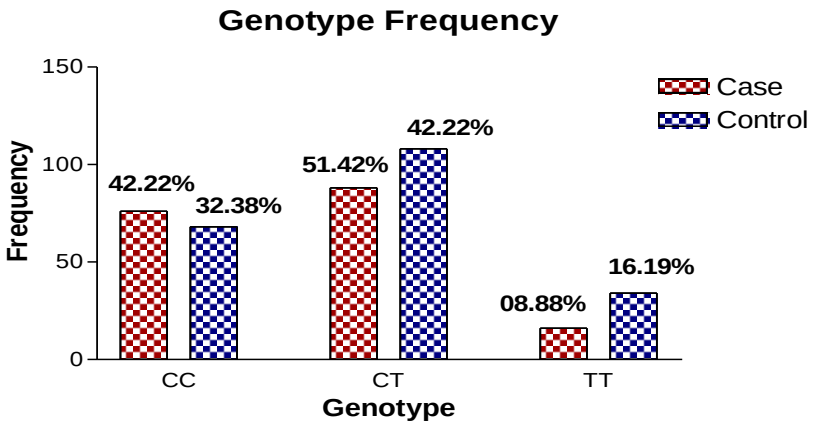
Table 3. Fisher Exact Test values of CD 14 Promoter Gene polymorphism

CD 14 Promoter Gene	CASE (N= 180)		CONTROL (N=210)		P Value	Odds Ratio (CI)
	N	%	N	%		
Genotype						
CC	76	42.22	68	32.38	0.0464*	1.526, (1.009 to 2.308)
CT	88	48.88	108	51.42	0.6846ns	0.9034, (0.6065 to 1.346)
TT	16	08.88	34	16.19	0.0340*	0.5050,(0.2686 to 0.9495)
Allele						
C	240	66.66	244	58.09	0.0147*	1.443, (1.077 to 1.933)
T	120	33.33	176	41.90		0.6932, (0.5173 to 0.9288)
Carriage Rate						
C						
T	164	61.19	176	55.34	0.1546ns	1.272, (0.9141 to 1.771)
	104	38.80	142	44.65		0.7860, (0.5647 to 1.094)

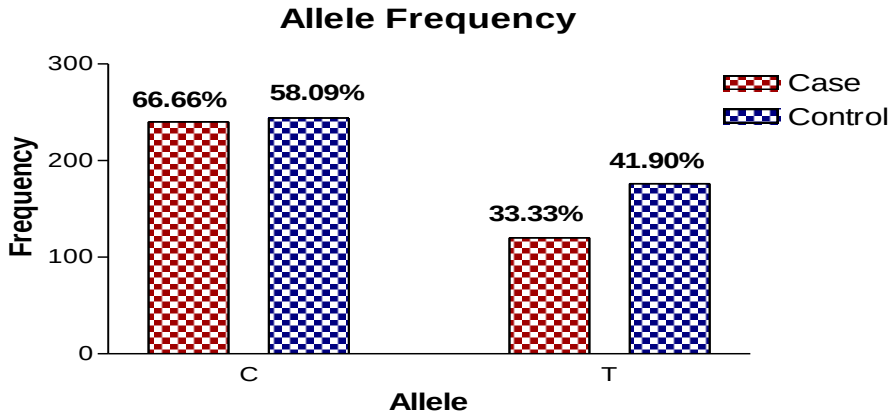
N – Number of individuals in study group.
%- Genotype allele frequency and carriage rate expressed in percentage.
(denotes the level of significant association between case and control).*

Graphical Representation of Genotype frequency, Allele frequency and Carriage rate

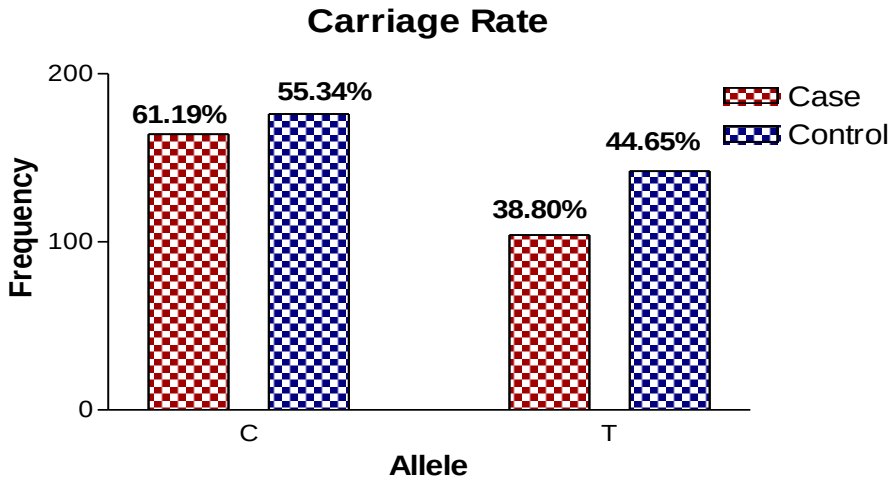
Comparative Graphical representation of genotypic frequency of CD 14 Promoter Gene is showing genotype distribution in patient and healthy population. In our population TC genotype (case-48.88%, control-51.42%) slightly low in percentage in comparison to CC genotype (case-42.22%, control-32.38%) whereas TT genotype (case-08.88%, control-16.19%) low in number. Allele frequency illustrate C allele (case-66.66%, control-58.09%) is high in comparison to T allele (case-33.33%, control-41.90%) . carriage rate C allele (case-61.19%, control-55.34%) is also high in compare to T allele (case-38.80%, control-44.65%).



Graph 1: Genotype Frequency of CD 14 Promoter Gene.



Graph 2: Allele Frequency of CD 14 Promoter Gene.



Graph 3: Carriage rate of CD 14 Promoter Gene.

Discussion

Anthropometric measurements were made and serum iron and ferritin levels determined in a group of Gambian children at the beginning of the rainy season and these findings were related to the malaria experience of the children during the following malaria transmission season. Susceptibility to malaria was not correlated with prior weight-for-age, height-for-age, weight-for-height or serum albumin, or with serum iron, serum iron binding capacity nor serum ferritin [13]. Thus, our findings do not provide any support for the view that poor nutritional status, as assessed by anthropometric measurements, or iron deficiency protect against malaria infection. Children who developed a clinical attack of malaria accompanied by a high level of parasitaemia tended to have a higher mean weight-for-age at the beginning of the rainy season than did children who had a clinical attack accompanied by a low level of parasitaemia, but the difference between groups was not statistically significant. However, they had a significantly higher mean serum ferritin level [14-18].

The association with nutrition with malarial infection revealed how anthropometric parameter influenced malarial infection. The complex relationship between malnutrition and malaria affects morbidity and mortality in children younger than 5 years, particularly

in parts of sub-Saharan Africa where these conditions occur together seasonally. Previous research on this relationship has been inconclusive [19,14]. This cross-sectional study is a secondary analysis of a cluster-randomized trial comparing treatment strategies for trachoma in Niger. The children aged 6–60 months residing in the 48 communities enrolled in the trial who completed anthropometric and malaria infection assessments at the final study visit The association between anthropometric indicators, including height-for-age z-score (HAZ) and weight-for-age z-score (WAZ) and indicators of malaria infection, including malaria parasitemia and clinical malaria. In May 2013, we collected data from 1,649 children. Of these, 780 (47.3%) were positive for malaria parasitemia and 401 (24.3%) had clinical malaria. Greater height may be associated with an increased risk of clinical malaria. In order to alleviate the burden of malaria in sub-Saharan Africa it is important to understand the impact of lifestyle variables for public health efforts to be effective at the level of the household [20-22]. Data trends are inferred from the patient's physical examination, and a food questionnaire detailing the daily diet of the patient. The average age in this sample was 34.66 years; the average BMI was 20.05 kg/m2 and the prevalence of malaria was approximately 8.5%. Data indicates that among those who had the most diverse daily diet, only 5% had malaria, while 9% of those who did not eat a daily diverse diet had malaria. Patients with a severely thin Body Mass Index (BMI) were found to be at a higher risk (12.8%) of having malaria, whereas the pre-obese and obese had no (0%) malaria. Data also indicated that with the average prevalence of worms being 7% in the sample, the severely thin manifested a proportion of 17%, with the pre-obese and obese manifesting no diagnoses of worms [23-27].

Our data anthropometric and life style factor revealed no any association to *plasmodium falciparum* malaria. We recruited 180 *P. falciparum* infected malarial patient and 210 healthy population in the respect of anthropometric measurements such as BMI (Body Mass Index) and WHR (Waist Hip Ratio). Comparative study of two parameters BMI and WHR between two groups are not showing any association. BMI factor is not significantly associated with malarial infection in both Women (P=0.2214) and men (P=0.7751) thus WHR is also not showing any association between malarial patient and healthy population in both Women (P=0.0811) and men (P=0.1106). We also collected data of life style factor such as smoking and drinking on both patient and healthy population. We have smoking (P=0.7242) and drinking (P=0.8740) data, suggest no association of these features with susceptibility to *P. falciparum* Malaria.

The relationship between malaria and obesity is largely unknown. This is partly due to the fact that malaria occurs mainly in tropical areas where, until recently, obesity was not prevalent. It now appears, however, that obesity is emerging as a problem in developing countries. To investigate the possible role of obesity on the host-parasite response to malarial infection, this study applied a murine model, which uses the existence of genetically well characterized obese mice [28]. The receptivity of obese homozygous ob/ob mice was compared to the receptivity of control heterozygous ob/+ lean mice after a single injection of *Plasmodium berghei* ANKA sporozoites. Both parasitaemia and mortality in response to infection were recorded. Results: The control mice developed the expected rapid neurological syndromes associated with the ANKA strain, leading to death after six days, in absence of high parasitaemia. The obese mice, on the other hand, did not develop cerebral malaria and responded with increasing parasitaemia, which produced severe anemia leading to death 18–25 days after injection. The observed major differences in outward symptoms for malarial infection in obese versus control mice indicate a link between obesity and resistance to the infection which could be addressed by malariologists studying human malaria [28-30].

CD14 is expressed principally by cells of monocyte/macrophage lineage and plays a pivotal role in the innate immunity to intracellular infections. Recent research findings have revealed an association between the CD14 gene promoter polymorphism and several major infectious diseases. For this purpose, 88 consecutive patients with tuberculosis (63 pulmonary, 25 extrapulmonary) and 116 control subjects were enrolled into a prospective study. We determined CD14-159 genotypes by polymerase chain reaction - restriction fragment length polymorphism analysis and also measured serum concentrations of soluble CD14 (sCD14) by using a quantitative sandwich enzyme immunoassay technique [31]. There was no significant difference in terms of genotype distribution between patients with tuberculosis (CC 18.2%, CT 48.9%, TT 33.0%) and controls (CC 12.9%, CT 50.9%, TT 36.2%) or between patients with pulmonary and extrapulmonary tuberculosis. Serum levels of sCD14 were significantly increased in patients with active tuberculosis compared to those with inactive tuberculosis and healthy controls ($p < 0.001$). However, levels of sCD14 were not associated with any genotypes of CD14-159. The genotyping findings of the present study do not support a role for the CD14-159C/T polymorphism in the development of tuberculosis, at least in the geographical region of central Anatolia. Significantly elevated serum sCD14 levels in patients with active disease reflect the importance of the mononuclear phagocytic system activation in tuberculosis [31-32].

The association between the cluster of differentiation 14 (CD14)-159C/T (rs2569190) gene polymorphism and susceptibility to acute brucellosis in an Iranian population. The study included 153 Iranian patients with active brucellosis and 128 healthy individuals as the control group. Genotyping of the CD14 variant was performed using an amplification refractory mutation system-polymerase chain reaction method. The prevalence of CD14-159 TT and CT genotypes were associated with increased risk of brucellosis [odds ratio (OR) = 1.993, 95% confidence interval (95% CI) = 1.07–3.71, P = 0.03 for CT; OR = 3.869, 95% CI = 1.91–7.84, P = 0.01 for TT genotype. Additionally, the minor allele (T) was significantly more frequently present in brucellosis patients than in controls (61% vs. 45%, respectively), and was a risk factor for brucellosis (OR = 3.058, 95% CI = 1.507–6.315, P = 0.01). The findings provided suggestive evidence of association of the CD14- 159C/T gene polymorphism with susceptibility to acute brucellosis in the Iranian Population [28]. Our statistical data from CD 14 Promoter gene polymorphism revealed that PCR amplification with specific primers gave 295bp product which was digested with *BsuR1* enzyme for 16 h at 37˚C. The wild-type genotype (CC) was digested at restriction

site. The wild type C allele having restriction site for *Hae*III enzyme, after mutation C allele concerted in to Mutant T allele where restriction site was disappeared. The Restriction site containing homozygous genotype (CC) was cut as a doublet of 155bp and 140bp. The heterozygous genotype (TC) was represented as 3 fragments of 295, 155, and 140bp. Statistical analysis containing Genotype frequency, Allele frequency and carriage rate of CD 14 Promoter gene. Case (Malarial patient) – Control (Healthy population) study in respect of Chi Square Test reveals that two type allele present in three genotype CC, TC, CC. Statistical analysis, between Malarial patient and healthy control reveals genotypic association as χ^2 (P Value) = 6.697, (0.0351*). Allele frequency is also significantly associated as χ^2 (P Value) = 6.048, (0.0139*) whereas statistical data of carriage rate suggest no association between case and control. Statistical data in the respect of Fisher Exact test between malarial patient and healthy population illustrates P-value and Odds ratio. Statistical analysis reveals TT genotype significantly associated with susceptibility to *P. falciparum* Malaria as P= 0.0340* whereas Odds ratio (0.5050, CI= 0.2686 to 0.9495) suggest protective genotype. Thus allele frequency is also significantly associated with malarial infection (Table no. 4.6). Odds ratio gives information about differences between two allele frequency and genotype frequency in case and control.

CD14 is a multifunctional receptor expressed on many cell types and has been shown to mediate immune response resulting in the activation of an inflammatory cascade, with polymorphism of its promoter (rs2569190) found to be associated with susceptibility to several diseases. In malaria infection, the CD14 gene demonstrated a pathogenic profile in regulating experimental cerebral malaria, with reports of elevated levels of soluble CD14 in serum of patients but no definitive conclusion.

Conclusion

We present a detailed analysis of genetic diversity of CD14 promoter gene (snp −159 C/T; rs2519190) polymorphism between a malaria-infected group and uninfected controls and its association with clinical parameters of disease **[14,19].**

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