Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

My research work dates back to my post graduate days (1981-1984) when I was selected as Senior Research Fellow under ICMR to develop newer diagnostic test for filariasis. That passion for research continued right through my career of 39 yrs. It can be broadly categorised as research on Bancroftian filariasis, followed by work on P falciparum malaria, and subsequently autoimmune disorders, notably SLE (systemic lupus erythematosus). Malaria and filariasis were the major public health problems of the state, and these diseases contributed enormously to the disease burden of parasitic diseases in the country. What followed in my career was an effort to connect the dots: exploring the link between lymphatic filariasis and malaria to systemic autoimmune disorders. Research in a state funded medical college is not easy. Besides dealing with the huge patient load, there are constraints of man-power and financial resources.

Research on Lymphatic filariasis:

I started working on Lymphatic filariasis which was the beginning of my scientific journey in 1981. I started by looking at the profile of clinical filariasis, and developing newer diagnostic tests, in an ICMR sponsored project. Diagnosing filaria infection by detection of mf in night blood was cumbersome and carried low sensitivity. But my first publication was the detection of B.malayi in coastal Odisha in 1989, which had not been detected earlier in the state **(Detection of new focus of Brugia malayi infection in Orissa. J Com. Dis. 21(1):39-40,(1989).** The clinical phenotypes of Bancroftian filariasis and B. Malayi are different although the treatment is identical. It was vital to identify them because the chronicity and manifestations differed. .But the focus was to develop a good, cheap and sensitive diagnostic test. I looked at antibodies like IgG and IgM in patients but it did not help to diagnose new cases or provide us with conclusive evidence. We tried culturing mf in the laboratory for excretory and secretory antigens which we believed would be useful for carrying out diagnostic ELISA, a more specific and sensitive method, but it was inconclusive as well. We started conducting drug trials between 1986 -1992 on alternative medicines for filariasis, under the aegis of ICMR and I worked as the Co-PI. There was no substitute for DEC, which was the only medicine available for treatment We tested an analogue of DEC called Centperazine, developed by CDRI, Lucknow. Although it showed promising results but was not found to be superior to DEC**. (Double blind clinical trial on Centperazine and DEC in Bancroftian filariasis. Ind J Med Res. 91:277-281. (1990).**

I started probing into other manifestations of filariais. The clinical manifestations of Bancroftian filariasis are sometimes atypical in endemic areas. And in the absence of a good diagnostic test they are often missed. Musculo-skeletal manifestations, allergic reaction and tropical pulmonary eosinophilia are a few notable examples. While exploring atypical clinical phenotypes I observed many patients presenting with haematuria/chyluria which could not be explained by other causes and but they responded to DEC therapy. On conducting kidney biopsy, we found immune complex deposits **(Renal involvement in bancroftian filariasis National Medi Jour of**

India; 4: 65-68, 1991). Immune complex deposits leading to glomerulonephritis in filariasis had never been reported before. Since acute lymphatic filariasis had strong inflammatory response, and at that point of time ,TNFα, was considered a very important pro-inflammatory cytokine, I believed it had a role in the pathogenesis of different clinical phenotypes and studied the levels of TNFα. It was very high in patients with acute adenolymphangitis and low in Mf carriers indicating a variable immune response depending on the stage of the infection.. It also correlated with the severity of the disease. In severe manifestation it was high but low in mild attack **(A role for tumour necrosis factor á in acute lymphatic filariasis. Parasite Immunology.18(8):421-424 1996)**

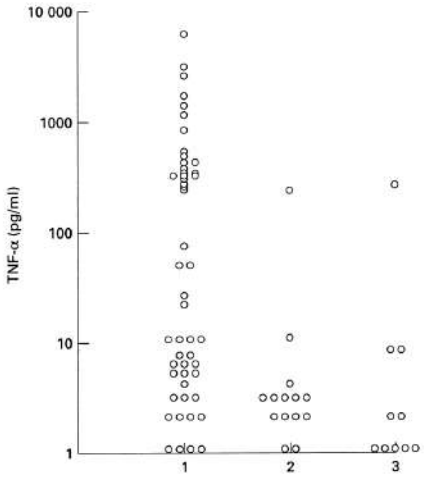


Figure 1 ELISA: Circulating TNF-α levels (pg/ml) in three groups of Bancroftian filariasis patients: 1-(AC) patients with acute filarial episodes ($n = 50$), 2-(C) microfilariae carriers ($n = 14$) and 3-(CH) patients with chronic manifestations of filariasis (lymphoedema/ hydrocele, $n = 10$).

Parasite Immunology

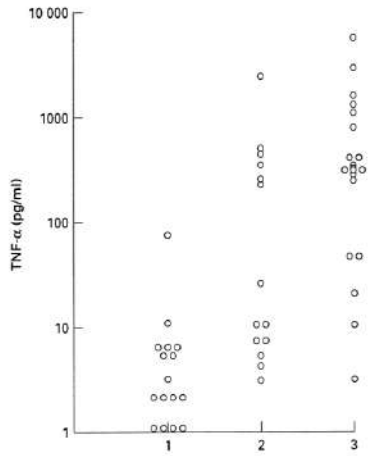
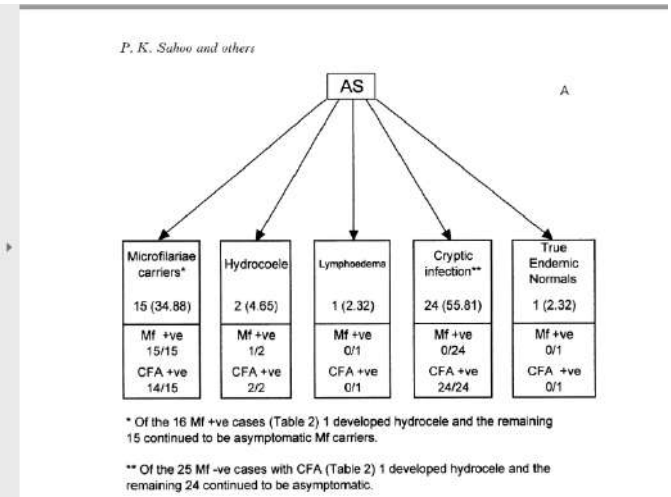


Figure 2 ELISA: Circulating TNF-α levels (pg/ml) in acute filariasis with different grades of severity: 1-mild (n=16); 2-moderate (n=14) and 3-severe (n=20); see text for details.

But the most intriguing aspect of Bancroftian filariasis was it natural history. It was presumed that once infected a human host carries the infection for long many years. But it was not known what was the basis of variable manifestations over time and what was the nature of this symbiotic relationship. Therefore, the natural history of human filarial infection leading to development of disease had been a subject of intense debate. The models proposed at that point of time was largely been based on cross-sectional data on microfilariae (Mf) and disease prevalence in filarial endemic areas. We did one of the longest follow-up seminal study, for a period of 13 years, on asymptomatic microfilaria carriers and endemic normal, to address this issue. The results suggested

that in Mf carriers, adult filarial worms persisted for several years, and that loss of circulating Mf with or without chemotherapy with DEC (single 12-day course) did not influence adult worm survival. Based on these findings a "static " and a dynamic model for the evolution of filarial infection was proposed and it has stood the test of time **.(A 13 year follow up study of asymptomatic microfilariae carriers and endemic normal in Orissa, India. Parasitology, 124: 191-201, 2002).**

It will be difficult to conduct these studies now after the commencement of MDA (Mass Drug Administration Therapy) annually in endemic areas for the elimination of filariasis from the country. I presume the elimination of these nematodes may have consequences notably an upsurge of autoimmune disorder**.**



This observation had a far reaching consequence on my subsequent studies on autoimmune disorders where I proposed a protective role for this nematode through immune modulation. Adult filarial worms survive for long periods of time, and a small percentage develop chronic disease. This work on filariasis was made possible by the availability of a highly sensitive and spectfic test at that time to detect CFA (Circulating Filarial Antigen).

The variable immune response in filariasis was evident from the clinical manifestations, TNFα response. And long term observation studies on infected hosts.We wanted to probe if filarial infection can modify severity of sepsis and malaria if they coexist. Both malaria and filariasis are endemic in Odisha. Prevalence of filarial antigenemia was significantly less in sepsis patients as compared to controls, On the other hand, levels of circulating filarial antigen were comparable in severe malaria cases and healthy controls. Observations of our study implied that successful control of filariasis could have adverse consequences on public health by increasing the incidence of sepsis(**Decreased prevalence of sepsis but not mild or severe P. falciparum malaria is associated with pre-existing filarial infection. Parasite Vectors.10;6:2013). Subsequently, I looked at the existence of filarial infection and its role in rheumatoid arthritis and systemic lupus erythematosus.**

Research on Malaria:

Malaria was a major public health problem in Odisha besides lymphatic filariasis. Nearly 84 % of cases were P. falciparum that contributed to lethal disease and a small percentage was contributed by P.vivax. Odisha had the highest number of P. falciparum cases in the country but concerted effort over the years under the National Control/Elimination Programme, the numbers have dropped drastically but still remains with low transmission potential in tribal districts.

I started working on malaria way back in 1990, looking at the various clinical manifestations (Five year retrospective study of hospitalized cases of falciparum malaria.J Int Med. 2(1): 12-14. 1990, Haematological and coagulation profile in acute falciparum malaria. JAPI. 40(9): 581-83,1992, I was interested in understanding the pathogenesis of severe malaria. Free oxide radical induced injury was a common presumption and we found that it was significantly high in acute falciparum malaria compared to vivax and healthy controls. Patients with high lipid peroxidation product had more complication and death. It was an association(Lipid peroxidation in acute falciparum malaria.IJMR,93;303-305, 1991). It was suggested that bacterial infection could be a trigger for progression of P falciparum infection to its severe form. We failed to demonstrate bacteraemia in blood culture from complicated or uncomplicated disease (Bacteraemia in adult patients presenting with malaria in India., Acta Trop. 123, 136-138, 2012). Then I looked into the immunological parameters to try to explain the basis of severe malaria. We looked at anti-malarial antibodies in the CSF of patients with cerebral malaria(CM). Changes in CSF closely reflects changes in the brain.88% of patients with CM had significant levels of anti malarial Ig G. It did not correlate with severity of coma but did with duration of coma.

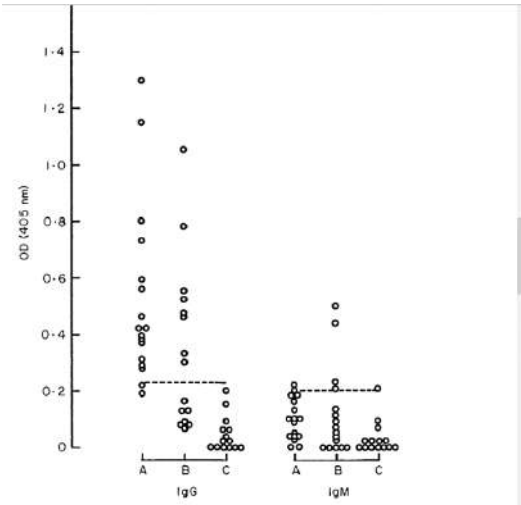


Fig. 1. ELISA. Malarial antibodies in CSF of cerebral malaria (A), presumptive cases of cerebral malaria (B) and normal controls (C). The dotted line indicates the cut-off value corresponding to the mean + 3 s.d. of control CSF for IgG and IgM.

Human cerebral malaria: characterization of malarial antibodies in CSF. Clinical and Experimental Immunology.86:19-21(1991)

Subsequently I looked into another auto-antibody in CSF called ant-gal, a naturally occurring autoantibody that works as a first line of humoral response in infection, to understand its relationship in CM. 70% of patients with cerebral malaria (CM) and Presumptive cerebral malaria (PCM) had high anti gal IgG antibodies. Since breach of CSF barrier in cerebral malaria it was presumed that the antibodies were synthesised intrathecally. Raised levels of anti-gal in CSF tend to indicate possible polyclonal stimulation of lymphocytes in the brain during CM.) **Human cerebral malaria: á galactosyl antibody in cerebrospinal fluid. (Trans Roy Soc Trop Med and Hyg; 86:132-133. 1992)**

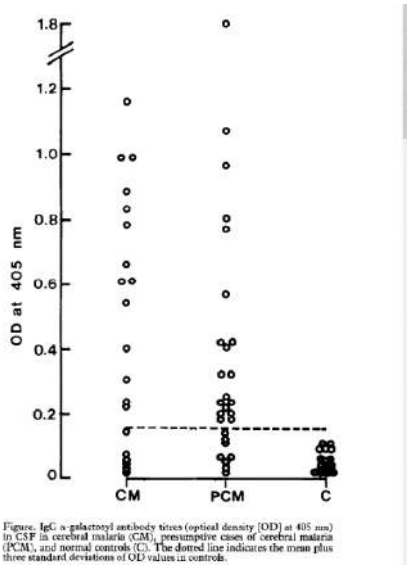
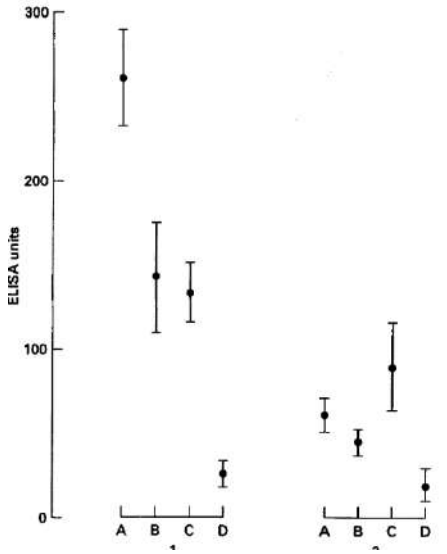


Figure. IgG a-galactosyl antibody titres (optical density [OD] at 405 nm) in CSF in cerebral malaria (CM), presumptive cases of cerebral malaria (PCM), and normal controls (C). The dotted line indicates the mean plus three standard deviations of OD values in controls.

Reports in the literature indicated that toxic malarial antigens consist of phospholipids and that antibodies to phospholipids (aPL) could inhibit such antigens in experimental systems and modify disease severity. I addressed this important observation with a series of experiments. Significantly raised levels of aPC (anti Phosphatidyl choline) were observed in infected individuals in comparison with controls. The IgG aPC levels were significantly more in those cases with cerebral malaria who recovered fully after quinine administration in comparison with fatal cases not responding to quinine therapy. Subgroup typing of IgG with aPC activity indicated lgG3 to be the predominant type, followed by IgG2, IgGl and IgG4. A significant inverse relationship between serum tumour necrosis factor-alpha (TNF-a) levels and IgGl antibodies with aPC activity was found, emphasizing the importance of aPC in modifying disease severity.This was a seminal observation(**A role for phosphatidyl Choline antibodies in human cerebral malaria. Clinical and Experimental Immunology.103(3):442-445.1996).**



Anti-phosphatidyl choline (aPC) antibodies in *Plusmodium fulcipurum* malaria; IgG (1) as well as IgM (2) aPC are shown in four groups of patients, as described in Patients and Methods; A, cerebral malaria survivors; B, cerebral malaria nonsurvivors; C, non-complicated *P. fulcipurum* malaria; D, normal control

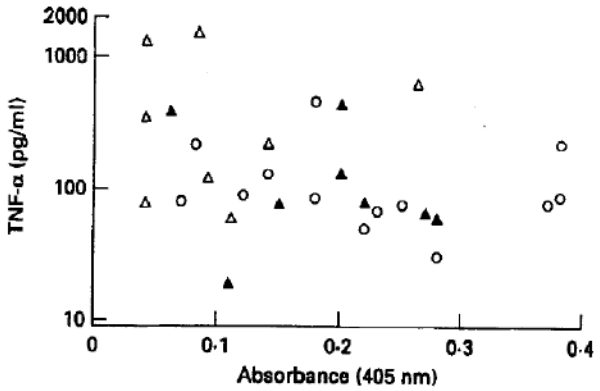


Fig. 2. Inverse relationship between circulating tumour necrosis factor-alpha (TNF-α) and IgG1 with anti-phosphatidyl choline (αPC) activity in *Plasmodium falciparum* malaria; ○, cerebral malaria survivors; △, cerebral malaria non-survivors; ▲, non-complicated *P. falciparum* malaria. ($r = -0{\cdot}365$, $P < 0{\cdot}05$.)

Some of the clinical manifestations were believed to have a strong association with TNF α, like hypoglycaemia in severe malaria. Plasma glucose was assessed with severe falciparum malaria at the time of presentation along with tumour necrosis factor-a (TNF-a). The lowest plasma glucose value was 3.38 mmol/l andnone of the patients had hypoglycaemia at admission. Plasma glucose values were not significantly lower in those with multiple organ dysfunction (MOD) than in patients with single organ dysfunction (cerebral malaria only) and in those who died compared with patients who survived. Conversely, TNF-a showed a good correlation with depth of coma and was significantly higher in patients who had MOD and those who died. There was no correlation between plasma glucose and TNF-a values. This was an important observation that negated the association between TNFα and hypoglycaemia provided evidence of an association between TNFα, severity of disease and mortality **(Plasma glucose and tumour necrosis factor alpha in adult patients with severe falciparum malaria.Trop Med and Int Health. 8(2):1-5.2003).**

R. Manish *et al.* **Plasma glucose and TNF-α in falciparum malaria**

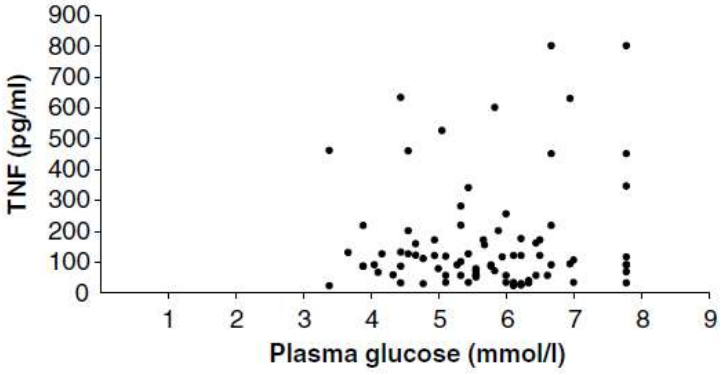
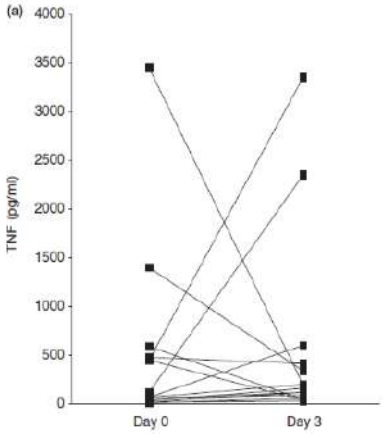
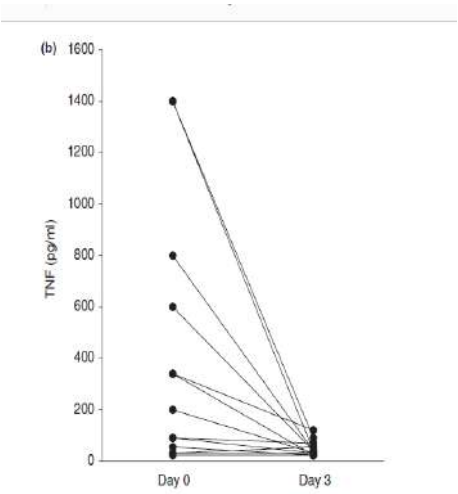


Figure 1 Scatter diagram of individual patients' plasma glucose and corresponding TNF-α levels at presentation.

We looked at the relationship between TNFα and complicated malaria by using a drug called Pentoxifylline which is haemorrheological agent with anti TNF activity as an adjunct therapy to quinine We enrolled patients with cerebral malaria divided them randomly into two groups. One group received quinine with pentoxifylline and the other only quinine IV for three days. TNFα decreased significantly in the group that received pentoxifylline but not in those who received only quinine. Since the number of patients in each cohort was small mortality could not be compared. This was a seminal paper that showed reduction of TNF α in cerebral malaria. **(Pentoxfylline adjunct improves prognosis of human cerebral malaria in adults.Trop Med and Int Health. 8(8):680-684 2003).**



Levels of TNF α in patients who received only Qunine (Day 0 and 3)



Levels of TNF α in patients who received Pentoxifylline plus Quinine(Day 0 and 3)

We looked at other factors that determines protection vs severity in P.falciparum infection. In an elegant study we looked at the ABO blood group of patients with severe, uncomplicated P. falciparum malaria and compared it with controls. Frequency of blood group 'B' was significantly higher in patients with severe malaria compared to the uncomplicated cases and healthy controls . Irrespective of the level of clinical severity, blood group 'B' was significantly associated with cerebral malaria , multi-organ dysfunction and non-cerebral severe malaria patients compared to the uncomplicated category. Prevalence of 'O' group in uncomplicated malaria and healthy controls was significantly high compared to severe malaria. Blood group 'O' was protective against severe malaria which was confirmed in a meta-analysis, while people with blood group 'B' are susceptible to severe disease**.(Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis. Malar J,10, 309. .2011)**

Blood group	Dead (n = 20)	Survivors (n = 104)	P value, OR (95%CI)
O	1 (5)	21 (20)	ref
A	2 (10)	18 (17)	0.59, 0.42 (0.03 to 5.12)
B	16 (80)	56 (54)	0.06, 6.00 (0.74 to 48.13)
AB	1 (5)	9 (9)	0.53, 0.42 (0.02 to 7.63)
Non O (A+B+AB)	19 (95)	83 (80)	0.12, 0.20 (0.02 to 1.64)

NOTE: Data are no. (%) of participants unless otherwise specified;
OR: odds ratio; CI: confidence interval

Association of ABO blood group in treatment outcome of patients with severe malaria

We analysed other factors in the RBC to predict outcome in severe malaria. CR 1 receptor is an important molecule on the surface of RBCs which is involved in the pathogenesis of the complicated disease. It helps in rosette formation which is one of the key pathogenetic factors that helps in plugging capillary flow in tissues of the body. The other factor is called cytoadherence which is mediated by other molecules. CR1 polymorphisms (intron 27 and exon 22) and blood group were typed in 353 cases of severe malaria (SM) [97 cerebral malaria (CM), 129 multi-organ dysfunction (MOD), 127 non-cerebral severe malaria (NCSM)], 141 uncomplicated malaria and 100 healthy controls from an endemic region of Odisha. The homozygous polymorphisms of CR1 intron 27 and exon 22 (TT and GG) and alleles (T and G) that are associated with low expression of CR1 on red blood cells, conferred significant protection against CM, MOD and malaria deaths. Combined analysis showed significant association of blood group B/intron 27-AA/exon 22-AA with susceptibility to SM (CM and MOD).(**Complement receptor 1 variants confer protection from severe malaria in Odisha, India. PLoS One. 7, e49420, 2012.**)

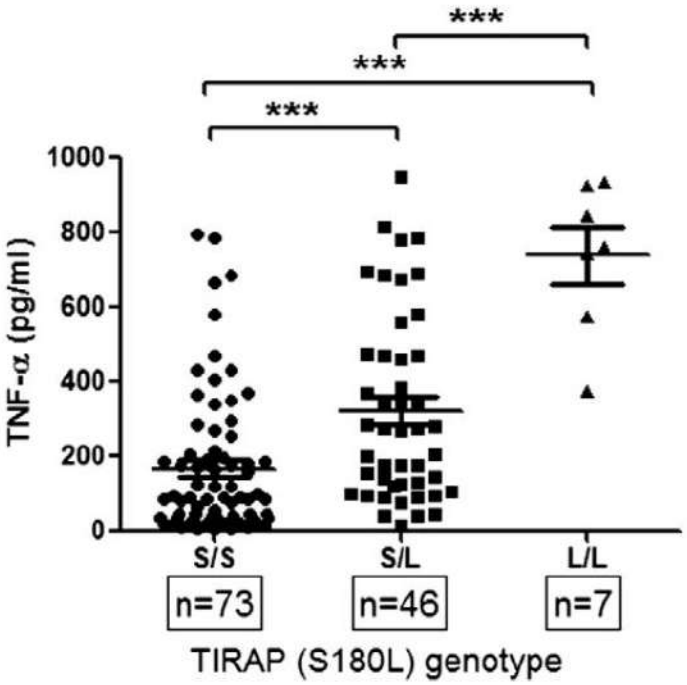
Haplotype analysis of CR1 polymorphisms in treatment outcome of P.falciparum malaria patients. The A-A haplotypes have the worst prognosis.

CR1 haplotypes	Survivors (n=450)	Dead (n=44)	P value, OR (95%CI)
G-T	42.06	21.26	ref, 1
A-A	36.62	61.03	**0.0007, 0.29 (0.14 to 0.59)**
G-A	16.38	15.11	0.19, 0.52 (0.20 to 1.30)
A-T	4.7	2.61	1.00, 0.91 (0.18 to 4.48)

NOTE: Data are shown in percentage. OR: odds ratio; CI: confidence interval.
doi:10.1371/journal.pone.0049420.t005

Toll-interleukin-1 receptor domain containing adapter protein (TIRAP) plays a crucial role in TLR2 and TLR4 signalling pathways. Glycosylphospatidylinositol (GPI), considered a toxin molecule of Plasmodium falciparum, interacts with TLR2 and 4 to induce an immune inflammatory response. A single nucleotide polymorphism at coding region of TIRAP (S180L) has been reported to influence TLRs signalling. we investigated the association of TIRAP (S180L) polymorphism with susceptibility/resistance to severe P. falciparum malaria in a cohort of adult patients from Odisha. TIRAP S180Lpolymorphism was typed in 347 cases of severe malaria (SM), 232 uncomplicated malaria and 150 healthy controls. Plasma levels of TNF-α was quantified by ELISA. Heterozygous mutation (S/L) conferred significant protection against MOD (multi organ dysfunction), NCSM (non-cerebral severemalaria) as well as mortality. Interestingly, homozygous mutants (L/L) had 16 fold higher susceptibility to death. TIRAP mutants (S/L and L/L) were associated with significantly higher plasma TNF-α levels compared to wild type (S/S). Any mutation that elevated TNF α levels caused increases disease severity and death. **(Heterozygous mutants of TIRAP (S180L) polymorphism protect adult patients with Plasmodium falciparum infection against severe disease and mortality. Infect, Genetics and Evolution, 43, 146-50, 2016).**

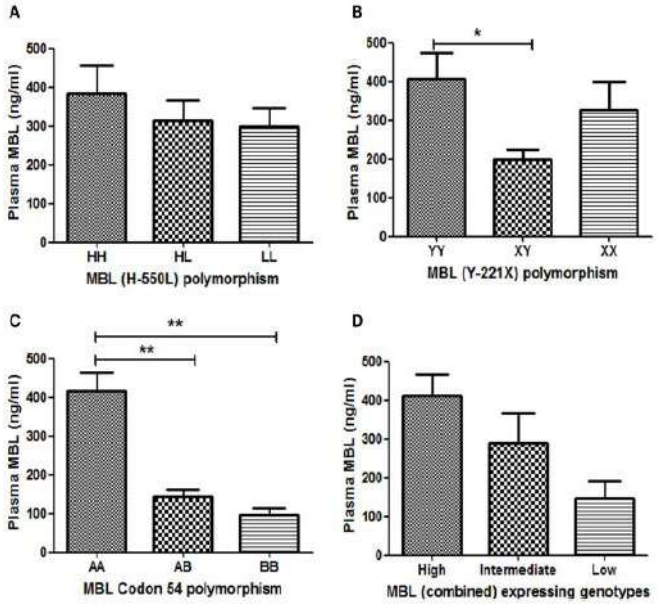
Association between TIRAP polymorphism and plasma TNFα levels In patients with malaria



There are several other molecules whose mutations can lead to severe malaria and an important protein of the innate immune system is MBL (Mannose Binding Lectin). Mannose binding lectin,a plasma protein protects host from virus, bacteria,and parasites. Deficiency in MBL level has been associated with susceptibility to various infectious diseases including *P.falciparum* malaria. Common MBL polymorphisms in promoter and coding regions are associated with decrease in plasma MBL levels or production of deformed MBL

respectively. We hypothesized that *MBL2* variants and plasma MBL levels could be associated with different clinical phenotypes of severe *P.falciparum* malaria. We studied common MBL polymorphisms (codon54,H-550L,and Y221X) were typed in 336 cases of severe malaria(SM) [94 cerebral malaria(CM), 120 multi-organ dysfunction(MOD),122 non-cerebral severe malaria(NCSM)]and131 un-complicated malaria patients(UM).Plasma MBL levels were quantified by ELISA. The observations of the present study revealed that *MBL-2* polymorphisms (codon 54andY-221X) and lower plasma MBL levels are associated with increased susceptibility to multi-organ dysfunctions in *P.falciparum* malaria.
(Frontiers in Microbiology, 6, 778. 20 ,015)

Association between MBL polymorphism and plasma levels of MBL



Multiple cytokines and other molecules have been implicated in the pathogenesis of malaria, notably severe malaria which is often lethal despite the best of treatment. Haem is an important constituent of RBC and parasite growth and it can affect disease outcome by several means. **Heme has been shown to play a role inthe pathophysiology of severe malaria in rodents, but its role in human severe malaria remains unclear. Circulating levels of total heme and its main scavenger, hemopexin, along with cytokine/chemokine levels and biological parameters, including hemoglobin and creatinine levels, as well as transaminase activities, were measured in the plasma of 237 *Plasmodium falciparum*-infected patients living in Odisha. Our results revealed significant increase in total plasma heme levels with malaria severity, especially for CM and malarial ARF. Analyses showed a significant correlation between total heme, hemopexin,interleukin-10, tumor necrosis factor alpha, gamma interferon-induced protein 10 (IP-10), and monocyte chemotactic protein 1 (MCP-1) levels. Our data indicate that heme, in association with cytokines and chemokines, was involved in the pathophysiology of both CM and ARF but through different mechanisms(Multifaceted roles of heme during severe falciparum infection in India. Infection Immunity 83(10):3793-9.2015)**

We looked at multiple cytokines to understand the pathways involved for various clinical phenotypes of P.falciparum malaria Severe malaria in India is characterized by high rates of complications like multi-organ dysfunction (MOD), which is not seen in African children, associated with acute renal failure and increased mortality. This study was conceived to identify cytokine signature signatures differentiating severe malaria patients with MOD, cerebral malaria(CM) and cerebral malaria with MOD(CM-MOD).

We determined plasma concentration of pro and ant-inflammatory cytokines and chemokines for all individuals using a multiplex assay. Of the 6 cytokines/chemokines tested, 19 increased significantly during malaria and clearly distinguished malaria patients from controls, sepsis and enchaphalitis who were taken as controls. High amount of IL17, IP 10 and IL10 predicted MOD, decreased IL 17 and MIP 1α separated CM-MOD from MOD. Increased IL 1p40 differentiated CM from CM-MOD. Most severe malaria patients with ARF exhibited high levels of IL17. We demonstrated that CM, CM-MOD and MOD are clearly distinct malaria associated pathologies. The IL17 pathway may contribute to malaria pathogenesis visa different regulatory mechanisms and may represent an interesting target to mitigate the pathological processes in malaria associated ARF.

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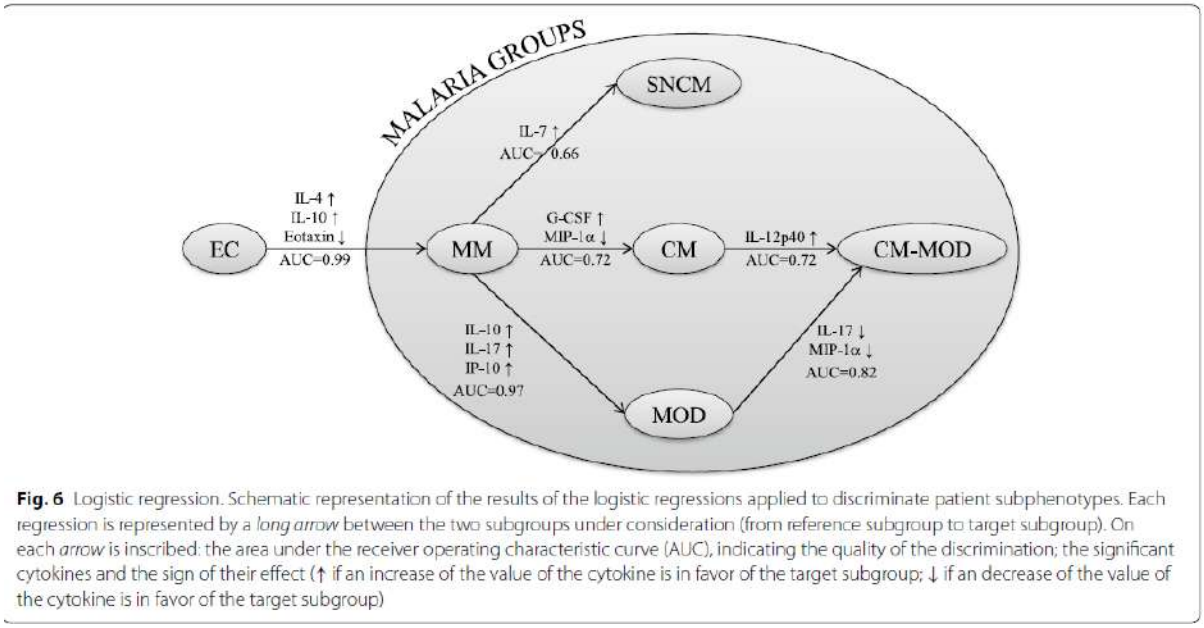


Fig. 6 Logistic regression. Schematic representation of the results of the logistic regressions applied to discriminate patient subphenotypes. Each regression is represented by a *long arrow* between the two subgroups under consideration (from reference subgroup to target subgroup). On each *arrow* is inscribed: the area under the receiver operating characteristic curve (AUC), indicating the quality of the discrimination; the significant cytokines and the sign of their effect (↑ if an increase of the value of the cytokine is in favor of the target subgroup; ↓ if an decrease of the value of the cytokine is in favor of the target subgroup)

Evidence of IL 17, IP 10,IL10 involvement in multi-organ dysfunction and IL 17 pathway in acute renal failure associated to Plasmodium falciparum malaria. J of Translational Medicine, 13:369, .(2015).

Erythropoietin has been another interesting molecule that plays an important role during the evolution of malarial infection with a possible role in treatment of CM. Erythropoietin (EPO) was recently suggested as a potential adjunctive treatment for CM. Administration of erythropoietin (EPO) has recently raised interest in the treatment of neurodegenerative diseases, including CM. It was likely that the beneficial impact of recombinanthuman EPO (rhEPO) on CM was concurrent to decreased production of the pro-inflammatorycytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α, and decreased recruitment of T lymphocytes within the brain We measured EPO levels in the plasma of well-defined groups of P. falciparum-infected patients, from the state of Odisha, with mild malaria (MM), CM, or severe non-CM (NCM). EPO levels were then correlated with biological parameters, including parasite biomass, heme, tumor

necrosis factor (TNF)-α, interleukin (IL)-10, interferon gamma-induced protein (IP)-10, and monocyte chemoattractant protein(MCP)-1 plasma concentrations by Spearman's rank and multiple correlation analyses. We found a significant increase in EPO levels with malaria severity and more specifically during fatal CM. In addition, EPO levels were also found to be positively correlated with heme, TNF-α, IL-10, IP-10 and MCP-1 during CM. We also found a significant multivariate correlation between EPO, TNF-α, IL-10, IP-10 MCP-1 and heme, suggesting an association of EPO with a network of immune factors in CM patients. **(Erythropoietin Levels Increase during Cerebral Malaria and Correlate with Heme, Interleukin-10 and Tumor Necrosis Factor-Alpha in India .PLoS One. e0158420, 2016).**

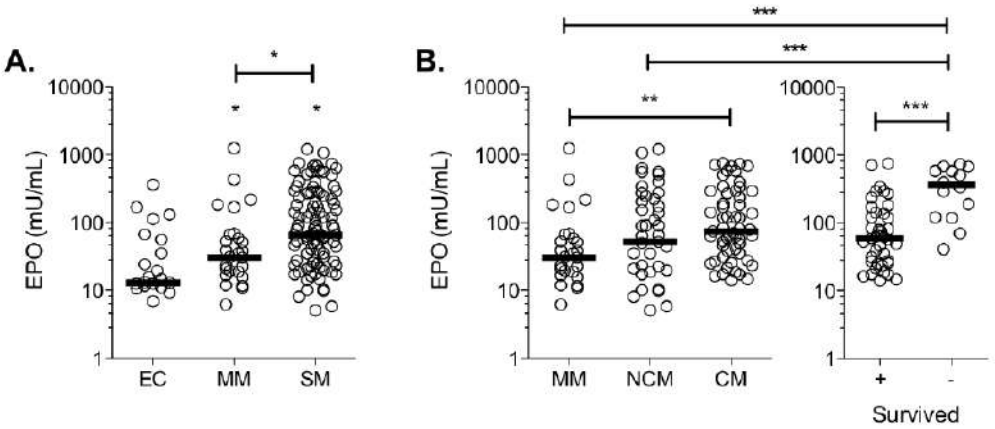


Fig 1. Erythropoietin levels increase during cerebral malaria and with severity of the infection. (A) Erythropoietin (EPO) levels were measured in the plasma of endemic controls (EC), mild malaria (MM) and severe malaria (SM) patients, and (B) in the SM sub-groups of non-cerebral malaria (NCM) and cerebral malaria (CM). (C) EPO levels were compared between CM patients who survived and those who did not. Significant differences with the EC group or between selected groups are indicated as the following: $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$.

doi:10.1371/journal.pone.0158420.g001

Research work on other Communicable diseases:

Dengue:

Dengue is the most rapidly spreading viral disease transmitted by the bite of infected Aedes mosquitos. The pathogenesis of dengue is still unclear; although host immune responses and virus serotypes have been proposed to contribute to disease severity. In this study, we examined the circulating dengue virus (DENV) and measured plasma levels of inflammatory mediators. Ninety-eight patients during a dengue outbreak in 2016 were included in the study. The presence of DENV was demonstrated by detecting NS1 antigen; IgM capture ELISA and serotypes were discriminated by type-specific RT-PCR and/or sequencing. Plasma samples were assayed for 41-plex cytokine/chemokines using multiplex Luminex assay. Eighty-five (87%) samples were positive by NS1/IgM capture ELISA/RT-PCR. All four serotypes of DENV were detected in this outbreak, with DENV-2 as the predominant type, seen in 55% of cases. Mixed infections were seen in 39% of subjects. Among the host inflammatory biomarkers, GM-CSF, IFN-, IL-10, IL-15, IL-8, MCP-1, IL-6, MIP-1_, and TNF-_ levels were significantly increased in dengue with and without warning signs, in severe

dengue patients in comparison to healthy controls. Four cytokines IFN-, GM-CSF, IL-10, and MIP-1_ correlated significantly with disease severity and could serve as potential predictor for disease severity. and guide in optimizing effective intervention strategies.

Covid and malaria:

During the recent Covid outbreak we probed into the possibility of protection from severe Covid in malaria endemic areas. We investigated the possible role of *Plasmodium* infection on coronavirus disease 2019 (COVID-19) infection or severity. Recent studies indicated that protozoan infections may offer some protection against various positive-strand RNA viruses. Prior exposure to plasmodia significantly suppressed chikungunya virus–associated pathogenesis, characterized by reduced viral load and improved joint inflammation. Furthermore, coinfections of a rodent *Plasmodium* strain and lactate dehydrogenase–elevating virus offered protection against experimental cerebral malaria and experimental autoimmune encephalomyelitis . Based on these observations on *Plasmodium* infection and positive-strand RNA viruses, we hypothesized that there could be a possible association between malaria and SARS CoV- 2 infection. To validate our observation, we investigated the prevalence of COVID-19 in the *Plasmodium falciparum*–endemic area of Odisha, Odisha is highly endemic for *P. falciparum* infection. We obtained the annual parasite index (API) of *P. falciparum* for the last 10 years (2010–2019) from the National Vector Borne Disease Control Program and COVID-19 infection
status in Odisha from the government of Odisha website, Annual Parasite Index (API) and COVID-19 data from30 districts were analyzed. A significant negative correlation was observed between 10-year average API scores and the number of COVID-19 cases detected.
(J.of Infectious Diseases,222 (9):1570-1571.,2020)

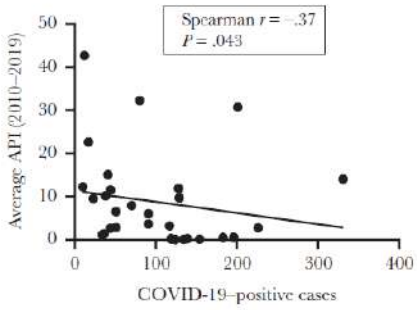


Figure 1. Correlation between the number of coronavirus disease 2019 (COVID-19) cases reported and the average annual malarial parasite index (API) during 2010–2019. Data from all districts of Odisha state were analyzed, and each dot represents 1 district. An inverse correlation was observed between API and number of COVID-19 cases reported through 19 June 2020 (n = 30).

Acute Encephalitis Syndrome(AES): Acute encephalitis syndrome (AES) is a major public health concern in India, causing febrile illness principally associated with viral infection. Bacteria-like scrub typhus and leptospirosis also cause acute febrile illness. Therefore, this study was conceived to address the possible etiological agents contributing to sporadic AES in a tertiary care center in Odisha, This was a prospective hospital-based study that enrolled 92 consecutive patients with clinically diagnosed AES whose blood/cerebrospinal fluid samples were tested for IgM antibodies to dengue, Japanese encephalitis (JE), herpes

simplex virus (HSV), Epstein-Barr virus (EBV), leptospirosis and scrub typhus. Significantly, antibodies to EBV in 23.59% and to JE in 29.34% cases were detected. Notably,32.60% and 12.0% of patients had IgM antibodies to leptospirosis and scrub typhus, respectively. This observation indicated an association of leptospirosis and scrub typhus infection in sporadic cases of AES which is vital because the management varies which can save many patients**.** **(Trans Royal Soc Trop Med and Hyg; 0: 1–3 doi /org:10.1093/trstmh/trab063,2021)**

Research on autoimmune disorders: SLE

There is a rise in prevalence and incidence of various autoimmune disorders across the world. The more serious ones are the systemic connective tissue disorders and inflammatory arthritis that constitute 10% of all rheumatological disorders. Among the them SLE (systemic lupus erythematosus) is the principal cause of mortality among young women afflicted with this disease. This disease has a genetic predisposition which gets triggered by environmental factors like UV light, viruses, chemicals, hormones and toxins. The clinical manifestations are heterogenous. Increased disease activity and infection are the major causes of death in the initial years. We looked at the clinical aspects of the disease.

Clinical studies:

The treatment of SLE is undergoing a transformation with the addition of new but costly drugs. We decided to probe the role of Vit D, an excellent immunomodulator, in SLE. Studies in various populations have shown an association between low vitamin D levels and higher SLE disease activity. We studied 129 patients of SLE, 100 matched controls and quantified Vit D3 levels as well as IFN α levels, the key cytokine that drives lupus pathogenesis. Plasma 25-OH vitamin D_3 significantly correlated in an inverse manner with systemic lupus erythematosus disease activity index (SLEDAI) scores, anti-dsDNA, plasma IFN-α, and levels of IFN-α gene expression. These results suggested an important role of vitamin D in regulating disease activity in SLE and the need to supplement vitamin D3 which is cheap and affordable**.** **(Arthritis Research and Therapy, 16(1):R49, 2014.)**

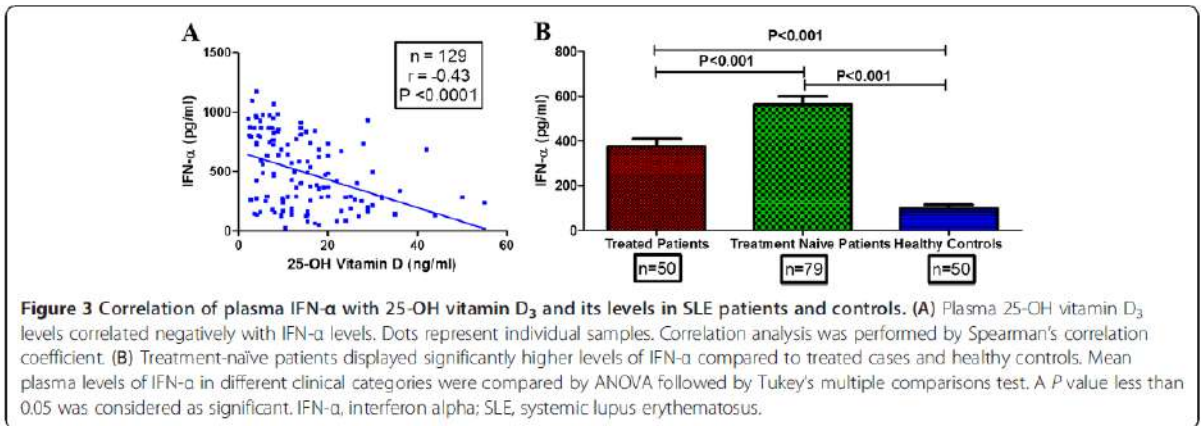


Figure 3 Correlation of plasma IFN-α with 25-OH vitamin D_3 and its levels in SLE patients and controls. (A) Plasma 25-OH vitamin D_3 levels correlated negatively with IFN-α levels. Dots represent individual samples. Correlation analysis was performed by Spearman's correlation coefficient. **(B)** Treatment-naïve patients displayed significantly higher levels of IFN-α compared to treated cases and healthy controls. Mean plasma levels of IFN-α in different clinical categories were compared by ANOVA followed by Tukey's multiple comparisons test. A *P* value less than 0.05 was considered as significant. IFN-α, interferon alpha; SLE, systemic lupus erythematosus.

Infection is a major cause of disease flare in lupus as well as one of the commonest causes of death. Sometimes both co-exist and it is necessary to differentiate both for proper management of the case. We did two studies to distinguish flare from infection. One hundred and fifty-two patients (152) were enrolled in one study and all had SLEDAI scores of more than 4, from which 70

had infection. Neutrophil–lymphocyte ratio (NLR) and C-reactive protein (CRP) were significantly elevated in SLE with infections than those with flare. The receiver operating characteristic curve (ROC) analysis revealed CRP, PLR, and NLR as important markers for predicting infections. NLR and CRP levels are affordable biomarkers to distinguish infections from flare, complications: a very important observation in the management of lupus.
(Clin Rheumatol. Jul 14. doi: 10.1007/s10067-022-06285- PMID: 35835900,.2022)

The second study Patients with SLE (SLICC criteria)presenting with fever between December 2018 and August 2021 were included. Neutrophil to lymphocyte ratio (NLR), NEUT-x, -y, -z indices, Erythrocyte sedimentation rate (ESR), C-reactive protein(CRP), C3, C4, anti-dsDNA antibodies, and procalcitonin(PCT) were tested. Based on the clinical assessment and laboratory data, the febrile episode was classified into infection, disease flare, or both. Among 168 febrile episodes in 166 patients with SLE 46 were due to infection, 77 due to flare, 43 due to both. High SLEDAI 2K (0.001), anti-dsDNA (p = 0.004), and low complements(C3, p = 0.001 and C4, p = 0.001) were characteristic of disease flare. whereas high total leukocyte count (TLC) NLR ,NEUT-x -y, -z ,CRP and PCT were observed with infection. A composite score of low cost and routinely available parameters like age, TLC, and CRPgives a good discrimination between infection and flare in a febrile patient with SLE.
Lupus. Sep;31(10):1254-1262. doi: 10.1177/09612033221112066,2022

Interleukin-6 (IL-6) and interferon-alpha (IFN-α) have been shown to have a major role in disease pathogenesis and they correlate with SLE disease. This study a investigated the significance of IL-6 and IFN-α levels in SLE pathogenesis. 70 SLE patients whofulfilled SLICC 2012 were enrolled. Levels of IL-6 and IFN-α were measured. IL-6 and IFN-α levels were elevated in SLE and they correlated with disease activity.
(Lupus. Aug;31(9):1094-1103, 2022)

Genetic Studies:

The genetic predisposition for SLE is believed to be around 30% based on twin and sibling studies. Multiple genes are implicated which are both MHC and non-MHC related. GWAS studies on Caucasians have revealed over 100 loci some of which are more prominent than other like PTP N22, STAT4, IRF 4,IRF7. But there are limited GWAS studies based on racial and ethnic groups. Many gene variants are being associated with lupus. One of them is MBL. Variants of MBL gene have been associated with autoimmune disorders. We explored common polymorphisms of MBL to look for its association with susceptibility to lupus and its clinical manifestations. Higher frequency of BB genotype and minor allele (B) was observed in patients of SLE compared to healthy controls MBL codon 54, H-550L, Y-221X polymorphisms and combined MBL genotypes contributed to plasma MBL levels. Prevalence of MBL low producer genotype (LXA/LYB, LYB/LYB and LXB/LXB) was significantly higher in SLE patients compared to healthy control. On analysis of clinical manifestations, MBL low producer genotype was significantly associated with autoimmune haemolytic anaemia. MBL is an important component of the complement pathway that helps in removing immune complexes . A low MBL level can be a factor in the onset of SLE **.(. Hum Immunol.74(1):114-9. 2013).**

MBL levels have also been shown to be high in SLE. We used that observation to assess if MBL can be utilised as a biomarker for disease activity. In a case control study SLE patients (93 females) and 67

age, sex, ethnicity matched healthy controls were enrolledPlasma MBL levels were significantly high in SLE patients compared to healthy controls. MBL levels were variable in different clinical categories of SLE. Lower levels were associated with musculoskeletal and cutaneous manifestations while higher and intermediate MBL levels were significantly associated with nephritis. Importantly, plasma MBL correlated with systemic lupus erythematosus disease activity index (SLEDAI) ,anti-dsDNA and negatively correlated with C3, markers of disease activity suggesting that it is a good biomarker**.(Mannose binding lectin: a biomarker of systemic lupus erythematosus disease activity. Arthritis Res Ther. 14, R218, 2012).**

The necessity for biomarkers in lupus is vital. There are conventional ones like anti dsDNA, C3,C4 but we have been looking at novel ones like MBL, sCD14 as indicated earlier. We probed the possibility of ferritin being used as a biomarker of disease activity. Ferritin is an acute-phase reactant that is elevated in various autoimmune disorders. Serum ferritin levels have been positively correlated with disease activity scores of RA and SLE. Further, enhanced levels of ferritin have also been reported in lupus nephritis. Seventy-six female SLE patients, diagnosed on the basis of revised ACR criteria, and 50 healthy females, age matched from similar geographical areas were enrolled. Serum levels of ferritin, IFN-a and IL-6 were quantified by enzyme-linked immunosorbent assay (ELISA). Clinical, biochemical, serological and other markers of disease activity (C3, C4 and anti-dsDNA) were measured. Serum ferritin levels were significantly higher in SLE patients compared to healthy controls. Ferritin levels positively correlated with SLE Disease Activity Index (SLEDAI) anti-dsDNA IFN-a and IL-6 and negatively correlated with C3 and C4. The observations provide evidence for ferritin being utilised as a biomarker in lupus(**Lupus. 24, 82-9.,2014)**

Lower CR1 expression has been associated with susceptibility to systemic lupus erythematosus (SLE). This study was the first of its kind to investigate the association of CR1 variants with development of SLE in a P. falciparum endemic population from Odisha. CR1 polymorphisms (intron 27 (A>T), exon 22 (A>G) and exon 33 (G>C)) were typed by PCR and restriction length polymorphism in 297 cases of female patients with SLE. and 300 age-matched and sex matched healthy controls from malaria endemic areas in Odisha, India. CR1 expression on monocytes was quantified by flow cytometry. The homozygous mutants of CR1 exon 22 (GG) and exon 33 (GG) and their minor alleles were associated with susceptibility to SLE. Patients with SLE who had the GG genotype of the exon 33 polymorphism had a 3.12-fold higher chance of developing lupus nephritis. The results of the study demonstrated that common CR1 exon variants were associated with diminished CR1 expression on monocytes and increased susceptibility to development of SLE and lupus nephritis in a malaria endemic area**.(Lupus Science and Medicine. 3(1):e000145,2016)**

Vitamin D deficiency/insufficiency appears to play a contributory role in the pathogenesis of SLE. Vit D works with a help of a VDR receptor whose mutation affects the functionality of Vit D. Vitamin D appears to have a regulatory role on disease manifestation and activity. Several studies

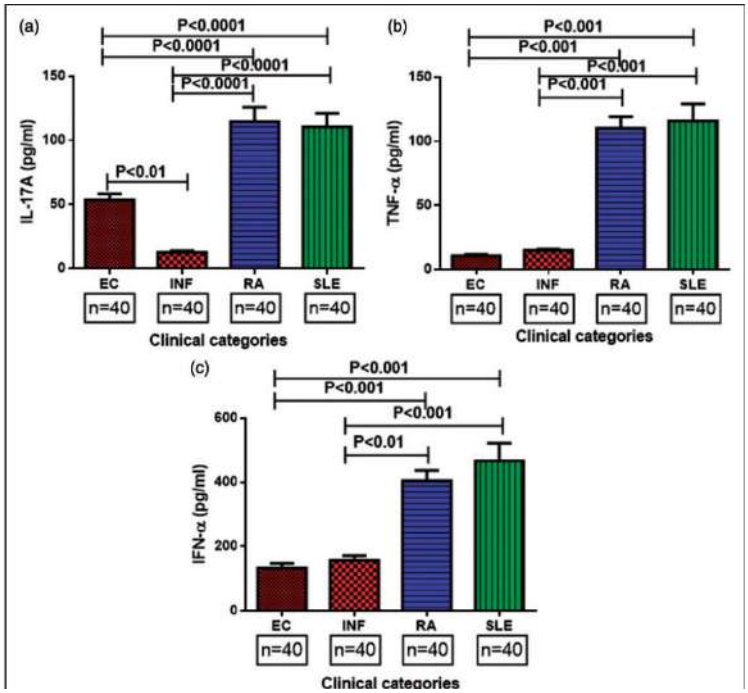
have demonstrated an association between VDR polymorphisms and susceptibility to SLE in different populations. In this study, we investigated the association of VDR polymorphisms with SLE. Female SLE patients (n = 331) who fulfilled the revised American College of Rheumatology classification criteria were enrolled along with 282 healthy controls from similar geographical areas. VDR polymorphisms (BsmI, ApaI, TaqI and FokI) were typed by polymerase chain reaction followed by restriction fragment length polymorphism. Plasma level of 25-OH vitamin D was quantified by enzyme-linked immunosorbent assay. FokI and TaqI variants were significantly associated with SLE in this cohort from Odisha.
(Int. Jour of Rheum. Dis 21(2):468-476.doi:10.1111/1756-185X.12345.2017)

We looked at anther important gene CD14 polymorphism for its association with lupus. CD14 molecule plays a crucial role in the innate immune response of the host in protection against various pathogens. The importance of soluble CD14 in autoimmune disorders has been described in different populations. However, the role of sCD14 in systemic lupus erythematosus (SLE) is poorly understood and the association of functional variants at the promoter region of the CD14 gene (_159C>T) needed to be defined with regard to susceptibility to SLE and disease severity.. Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method was used to genotype CD14 (C-159 T) polymorphism. Plasma levels of IFN-a, TNF-a, and sCD14 were quantified by enzyme-linked immunosorbent assay (ELISA). Prevalence of mutant genotypes (CT and TT) and minor allele (T) of CD14 (C-159T) polymorphism was significantly higher in SLE cases compared to healthy controls. Lupus nephritis patients had a higher prevalence of homozygous mutants (TT) and mutant allele (T). SLE patients displayed significantly increased plasma sCD14, TNF-a, and IFN-a levels in comparison to healthy controls. sCD14 levels correlated positively with SLE disease activity index-2K (SLEDAI-2K) scores and 24 hours proteinuria. sCD 14 can be utilised as a disease activity marker in lupus.
(Lupus, 30(2),219-227,2020)

Research on the interaction of parasitic diseases with SLE:

Based on a seminal paper that demonstrated complete reversal of CIA (collagen induced arthritis in an animal model with nematode protein ES 62, I looked at the possible link, considering Odisha as endemic for Bancroftian filariasis and Malaria.The study was carried out to explore the relationship between SLE and filariasis, Female patients(n319)residing in filarial endemic area were enrolled along with 63 healthy controls. They were subjected to Og4C3 ELISA test, a sensitive and specific test for circulating filarial antigen. While 42% demonstrated filarial infection none was detected in SLE suggesting a possible protection association **.(Lupus. 23, 1553-4,014).**

We explored further for a mechanistic cause for protection. A total of 160 individuals, 40 each of endemic normal, filaria infected cases, SLE and RA patients residing in filarial endemic areas were enrolled. Plasma levels of IL17A, IFNα and TNFα were quantified. SLE and RA patients demonstrated significantly higher levels of IL17, IFN and TNF. Interestingly IL17A were significantly low in filarial infected cases IL17 levels correlated inversely with CFA . Filaria infection possibly works through suppression of IL17 pathway.



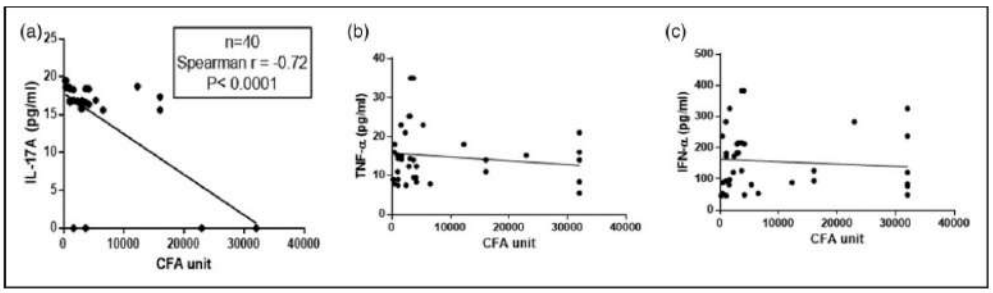


Figure 3 Correlation of plasma parameters with circulating filarial antigen levels (CFA). CFA levels were significantly correlated with plasma levels of IL17A (a) but not with TNF-α (b) and IFN-α (c). Dots represent individual sample. Correlation analysis was performed by Spearman correlation coefficient. A *p* value less than 0.05 was considered significant. IL-17A: interleukin 17A; TNF-α: tumour necrosis factor alpha; IFN-α: interferon alpha.

In an identical study we looked at RA patients in a filarial endemic area for a possibl protective role.Female patients(n – 207) were enrolled along with 222 matching controls. CFA analysis was carried out. 40% normal controls demonstrated filarial infection while no RA patient had CFA positive.

(Arthritis and Rheum. 65/5/ 1402-3,.2013)

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine associated with autoimmune and infectious diseases. Importance of TNF-α in P. falciparum malaria and systemic lupus erythematosus (SLE) have been demonstrated. A total of 204 female SLE patients and 224 age and sex matched healthy controls were enrolled in the study. Three hundred fourteen P. falciparum infected patients with different clinical phenotypes were included. TNF-α polymorphisms (G-238A & G-308A) were genotyped by PCR-RFLP. Plasma levels of TNF-α was quantified. TNF-α (G-238A and G-308A) variants were associated with higher plasma TNF-α. TNF-α (G-238A & G-308A) variants are associated with higher plasma TNF-α levels in SLE patients residing in malaria endemic areas and could be a contributing factor in the development of SLE and susceptibility to severe P. falciparum malaria.

(Nature Scientific Reports.9:11752.,2019)

(Dr Bidyut Kumar Das)