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-A.K. Goel and D. Kumar

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CHALLENGES FOR MALARIA CONTROL IN TRIBAL AREAS OF INDIA

Tazeen Iram Kareemi,[a] Praveen K. Bharti [b] and Neeru Singh[c]

Abstract: *Despite decadal efforts for achieving effective control, malaria remains a leading cause of morbidity and mortality in India. The problem of malaria is aggravated in tribal areas of the country that contribute a significant number of malaria cases to the national total. Control of malaria in the tribal areas is imperative for containment of malaria in the country, but it is hindered by multiple factors such as logistical and operational problems, typical geography and topography of the region, absence of proper diagnostic facilities, emerging drug resistant parasites, and year-round prevalence of vectors becoming increasingly resistant to insecticides.*

INTRODUCTION

India presently accounts for 70% of malaria cases in the South East Asia Region.[1] A major proportion of this burden is contributed by tribal population inhabiting malaria-endemic regions of the country. There are 104 million tribals[2] of various ethnic origins that contribute 46% of total malaria cases and 47% of all malaria deaths in India.[3] As the tribal population constitutes~8% of the country's population, these communities bear an unduly heavy burden of the disease. Control of malaria in this population would certainly help to change the overall scenario of malaria in India. However, effective malaria control in these settings is hampered by multiple challenges. A comprehensive discussion of the major challenges that affect malaria control in the tribal settings of India is presented with an objective to shed light on the hitherto unseen problems and to assist in planning and implementation of control strategies.

Logistic and Operational Problems: Major Setbacks in Malaria Control

Control of malaria is logistically difficult as majority of the tribal population tend to inhabit remote areas, which are difficult to access due to the hilly terrain and forest area containing numerous intersecting streams.[4] These streams are prone to frequent floods, which disrupt communication for several months. As the water level subsides after the rainy season, numerous breeding sites suitable for mosquito breeding proliferate. These breeding sites are also covered by dense aquatic vegetation, which makes the vector control difficult.[3] Moreover, the villages are scattered whereas health care facilities are inadequate with poor infrastructure and are understaffed.[5]

Various factors of human ecology and behavior also contribute to the overall burden of malaria.[6] People are mostly illiterate with little or no knowledge about cause and cure of malaria. They prefer to

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approach traditional healers and guniyas for treatment who are available locally in their communities.[7] It is only when the disease progresses to severe forms or when the symptoms worsens, that they seek formal health care services. But, as soon as the ailments subside, they discontinue the treatment leading to maintenance of parasite reservoir and emergence of drug resistance. The treatment of malaria is further made difficult due to the constant migration of people to new regions for seeking employment.[7] Cultural habits also impede control programs such as frequent mud plastering of walls[5] that negate the influence of spraying operations. Bed nets are also ineffective in remote tribal villages due to outdoor life and forest-based economy of the tribals.[8] All these situations tend to make malaria control difficult in the tribal belts.

Malaria Diagnosis: Pitfalls and Payoffs

An important element for effective surveillance and management of malaria is early and accurate diagnosis. Malaria diagnosis involves confirmation of malaria parasite either by (i) microscopic examination of blood smear or (ii) use of Rapid Diagnostic Tests (RDTs).[9] While, microscopy is considered as the gold standard for diagnosis of malaria, it requires expertise, time and is impractical for use in remote field settings. Additionally, microscopy is a major problem in hospitals and health centers that are already understaffed. Rapid Diagnostic Tests provide an alternative method that are simple to use, quick and can be used even by grass root workers to provide on-the-spot diagnosis.[9] The performance of RDTs for malaria diagnosis has already been evaluated in the field settings of tribal areas in Central India.[10] However, the efficacy of this test is compromised owing to its inability to detect low parasite densities[11], false positive results due to persistence of antigens in blood after treatment[12] and false negative results due to gene deletions or variations.[13,14] Further, inadequate use of RDTs due to lack of proper training has been reported.[5] Inaccurate diagnosis has a significant effect in increasing morbidity and mortality, intensifying drug pressure leading to development of resistance, maintaining the reservoir of disease and facilitating transmission.

Malaria Vectors: Constraints in Control

Malaria transmission in India is carried out primarily by six vectors, viz., *Anopheles culicifacies, An. fluviatilis, An. dirus, An. minimus, An. sundaicus* and An. *stephensi*.[15] The former five are primarily involved in transmission in tribal dominated regions, while *An. stephensi* is an urban vector. *An. culicifacies* is the principal vector found in the plains of rural India and contributes to about 60-70% of all malaria cases[14], which accounts for the bulk of outbreaks and epidemics.[16,17] A study conducted in Central India reported the year round prevalence of *An. culcifiacies* and change of its resting preference from indoors (endophilic) to outdoors (exophilic).[18] This change in behavior is of particular concern as it may impact the overall effectiveness of control measures that involve insecticide spraying inside the house. *An. culicifacies*

is widely distributed in India and its control is a menace. Each year, a large portion of malaria control budget is spent on control of this vector; still, its control below a critical level of transmission remains a formidable challenge.[9] Another important vector in the mainland of India is *An. fluviatilis*, which is responsible for 15% of malaria cases annually.[15] The control of this vector is difficult due to its abundant distribution in foot hill sand forests that are poorly accessible.[19] *An. sundaicus* is a brackish water species that contributes to a few thousand cases annually.[19] Control of this mosquito is feasible.[9] DDT sprayings in the past wiped it out from the mainland of India[6] and now it is confined only to Andaman and Nicobar Islands.[15] *An. dirus* and *An. minimus* are the vectors in the northeastern states of India. Once eliminated under the pressure of DDT, *An. minimus* resurfaced after a period of 45 years and now contributes to about 50 % malaria cases in the northeast.[15] *An. dirus* is one of the most efficient vector, contributing nearly all the remaining 50% cases in the region.[15] Its control is extremely difficult due to its occurrence in deep forested areas.[19]

Control of malaria depends largely upon the use of chemical insecticides for vector control. Insecticides employed are DDT, malathion and synthetic pyrethroids. These are used in Indoor Residual Sprays (IRS), whereas, synthetic pyrtehroids are also used for impregnation of bednets. Use of insecticides holds a history of enormous success that nearly eradicated malaria from the country in the past.[19] However, the effectiveness of insecticides is held back due to the development of resistance in the vectors. Resistance to DDT and malathion is widely reported[20, 21] while increasing reports of development of resistance against pyrethroids[22, 23], the only class of insecticide used for impregnation of bed nets, threatens the malaria control program.

Drug Resistance in Parasites: an Incessant Battle

A major concern in malaria control is the emergence of resistance in *Plasmodium* parasite against antimalarial drugs. *P. falciparum*, the deadliest parasite, is the reason behind changing drug policies worldwide due to the rapid development of resistance to all the classes of first-line antimalarial drugs. In India, resistance to chloroquine (CQ) was first detected in Assam in 1973, which then spread to the mainland by moving from Northeast through Odisha to Madhya Pradesh and is now widespread in the country.[9] This was followed by the emergence of Sulphadoxine-Pyrimethamine (SP) resistance in 1979, which was the same year of its introduction in drug regime.[24] With the increasing reports of treatment failures and deteriorating malaria condition, drug policy was changed to Artemisinin Combination Therapy (ACT) as the first-line antimalarial drug in 2010.[25] ACT is an effective therapy for treatment of uncomplicated *P. falciparum* malaria. However, resistance to this therapy has been reported in the area near the Thailand-Cambodia border with reports of reduced susceptibility in Northeast India.[9] With no other treatment alternative

in the antimalarial armory, the biggest threat today is the spread of ACT resistance to other parts of the country, as was seen in the case of CQ and SP.

CONCLUSION

In conclusion, the fight against malaria would require fine tuning the existing strategies and shedding light on the hitherto unseen problems of malaria control. The review has discussed key challenges for controlling malaria in the tribal dominated regions. An integrated approach towards malaria control is suggested that include (i) community awareness towards malaria and its control, (ii) strengthening laboratory services and health care facilities for early and accurate diagnosis and prompt treatment, (iii) vector control based on both chemical and bioenvironmental methods, (iv) source reduction by the removal of vector breeding sites and (v) regular monitoring of insecticide resistance in vectors and drug resistance in parasites. At the same time, effective surveillance and proper case reporting is also equally important since an underestimation of the true burden of malaria in India has been reported at several places.[5,19,26,27] The Government of India has targeted a Malaria-free India by 2030,[28] mainly by aiming to provide better logistic support, diagnostic tools, improved protective measures and effective antimalarial drugs. While the country is still in the malaria control phase,[19] years ahead will require extensive efforts and sensible measures to attain the goal of Malaria-Free India.

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TROJANS IN TRIBAL HEALTH

Himanshu S. Chandel,[a] Praveen K. Bharti [b] and Neeru Singh[c]

Abstract: *The tribes in India mostly reside in densely forested areas lacking basic health and infrastructural facilities as compared to urban areas. The health issues of tribes in these areas require special attention due to various socio-economic challenges. The extensive studies on diseases encountered in urban areas like diabetes, hypertension and infectious disease such as malaria should also be done thoroughly among tribal populations.*

INTRODUCTION

India is a habitat to various indigenous tribes, who comprise nearly 10% of the entire national population. Average state tribal population exceeding the national average of 8.61% is seen in the state of Madhya Pradesh (25.7% of state population), Chhattisgarh (30.6%), Orissa (22.8%), Jharkhand (26.2%), Sikkim (33.8%), Arunachal Pradesh (68.8%), Meghalaya (86.1%), Tripura (31.8%), Manipur (35.1%), Mizoram (94.4%) and Nagaland (86.5%).[1] Due to their varied and distinct socio-economic, educational, cultural and genetic makeup every national health policy planned or implemented has provided special facilities or status for all these tribal populations.

Despite the rapid development in the national healthcare policy and facilities, tribal populations in India still suffer from the neglected tropical diseases like Helminth infections, Visceral leishmaniasis, Lymphatic filariasis, Leprosy, Dengue and Japanese Encephalitis, that have been eliminated or are near elimination in developed countries.[2] While, bold questioning of the challenges faced by the affected population and then collating information from the respondents remains the two critical issues involved in dealing with any disease. The occurrence of misinformation between these two processes is designated as a real 'Trojan'. These Trojans in tribal health are diseases that have escaped attention or are overlooked due to limited scrutiny by health authorities. Although, cardiovascular diseases, cancer and diabetes are among the top ten most death-causing diseases in the world[3], the study and status of urban diseases like hypertension, cancer, and diabetes in tribal population is very limited. [4,5,6] In India, the alarming rise of these diseases in recent decades is a grave issue of concern and needs to be discussed at length.

A) Genetically-linked Heamoglobinopathy

In 2000, it was estimated that 45 million carriers (heterozygous) for haemoglobinopathies with allele frequency varying from 3-17 % was present among the Indian population.[7] The prevalence of sickle cell disease is estimated (with 10-40%) to be the highest among all haemoglobinopathies in India.[8] While,

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other haemoglobin disorders like α-thalassaemia, β-thalassaemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency were also reported, it was observed that sickle cell disease (SCD) was highly prevalent among the tribal populations.[9] The study conducted in Vidarbha region (Maharashtra and Chhattisgarh states) showed that sickle cell trait was more frequent in the Pardhan (20.31%) and the Marar (16.10%) communities, followed by Dhiwar (11.90%), Gond (11.89%) and the Mahar (11.81%). In contrast, β-thalassaemia was detected largely among the Sindhi (9.27%) and the Pardeshi (6.6%) populations[10] and less frequently among the tribals. These differences in disease prevalence bear a high impact on the urban and tribal populations since endogamous culture is commonly practiced in the tribal communities and is one of the possible reasons for the amplification of certain genetic diseases. More studies with illustrative data to identify the exact nature of these genetic diseases in tribal populations are essential. Currently, joint efforts are being made to identify these causes by various ICMR institutes in collaboration with the state health authorities.

B) Asymptomatic Malaria

One of the biggest challenges in malaria control and elimination are asymptomatic infections. The asymptomatic malaria cases are not easily detected in patients due to lack of symptoms of malaria known to them. This could be due to the deficient knowledge about discomfort and symptoms for these diseases in endemic areas. These individuals serve as a reservoir and easily escape all symptom-based malaria control programmes. To overcome such asymptomatic malaria cases, vaccination or active surveillance strategies are required. It was observed that total 3% of tribal population from ten states contribute nearly 14% of all malaria cases in the country.[11] This constitutes an alarming situation and suggests that malaria intervention approaches are restricted or limited in these tribal dominated areas due to various reasons. The other major challenge relates to mixed infection of different *Plasmodium* species reported from different states. The diagnostic tools used for malaria detection are mostly microscopy-based techniques widely regarded as the benchmark and RDTs are used usually in endemic areas. Recently, it was observed that mixed infection of different *Plasmodium* species was detected by PCR, but was initially reported as mono-infection using microscopy and/or RDTs.[12] These findings suggest that improper detection and treatment leads to failure of malaria intervention programmes.

C) Diabetes

The number of people affected with diabetes is increasing rapidly in India at an alarming rate. In 2000, total 31.7 million people were reported suffering from diabetes in India.[13] This was followed by 20.8 million cases from China and 17.7 million cases from United States of America (USA), with expected rise in cases to 79.4 million from India, 42.3 from China and 30.3 from USA by 2030.[14]

Diabetic patients are also increasingly found not only in urban but also in the rural populations of India.[13] A phase-I study conducted during 2008-2011 by ICMR institutes found that diabetic patients from rural population of Tamil Nadu, Maharashtra and Chandigarh were as high as those found in Jharkhand alone, even though Jharkhand reported very low self-reported cases. In Chandigarh the prevalence of diabetes was equally present in both rural and urban populations.[15] The tribal areas also reported prevalence of diabetes equal to those detected in rural areas of Telangana state. Further, 5.2% diabetes cases were found in tribes belonging to Jhunjhunu district of Rajasthan.[16] With estimated 1-10% diabetes cases present in different tribal populations[17], the increase of such diseases in rural population suggests that there could be significant prevalence of diabetes in all Indian tribes. It is also important to know that the risk of Diabetes mellitus increase in people having hypertension.[18]

D) Cardiovascular Diseases

Cardiovascular diseases are recognised as the top death causing disease during 2010-2013 in the world and in India.[3,8] In India, cardiovascular diseases are the major cause of death in both urban and rural populations.[19] In 1990, 2.3 million deaths due to cardiovascular diseases were reported in India and were estimated to double in number by 2020.[20] With hypertension cases increasing in middle and low income countries, the estimated number of affected people will increase from 118 million in 2000 to 213 million by 2025.[21] A study conducted in the tribal areas of Mandla District in India (Madhya Pradesh) showed 22% pre-hypertension and 32.7% hypertension cases in tribal people.[22] Since, cardiovascular diseases are generally associated with hypertension, the changing lifestyle and job opportunities in recent decades could increase the number of people affected with hypertension. An estimated 33% of urban and 25% of rural Indians are hypertensive, the awareness and treatment of hypertension is only restricted to one-fourth population in rural areas.[23] As there are very limited studies and data available on tribal populations affected from cardiovascular diseases, the highest number of deaths due to these diseases augments the need for regular monitoring and prevalence studies among different tribal populations.

E) Malignancy and Other Neoplasms

There are approx. 3.7 million known cancer cases in India with 1 million new cases and 0.68 million deaths occurring during 2012.[24] It is the fourth-most death-causing disease in urban areas, whereas, in rural areas of India, it is positioned at number five.[25] The global positions of the most prevalent cancer in men are led by prostate cancer, followed by lung and colorectal cancer, while lung cancer is the leading death-causing cancer followed by prostate cancer and colorectal cancer. Among women, breast cancer is the most prevalent cancer with lung and colorectal cancer following in second and third place. Most deaths are reported due to lung cancer.[25] The detection rate of oral cavity and oesophageal cancers are high

in India as compared to other countries of the world. The leading cancer-susceptible organs in Indian men are the lips, oral cavity (buccal mucosa), lung and stomach cancer, while in the case of women; breast cancer is followed by cervix and colorectal cancer.[24] Although, efforts are ongoing for detection of cancer of buccal mucosa, tongue, gastric, colorectal, gallbladder, prostrate and uterus in urban areas of different states, but very limited study observed in tribal areas of central India.[26]

Tobacco is well known causative agent of cancer especially buccal mucosa. Further, use of tobacco is mostly found among individuals from rural areas and tribal people.[5] Buccal mucosa cancer is less frequently observed in developed countries; this could be due to less use of chewable form of tobacco as compare to smoking. It is found that in India, more than half of male patients and one fifth of female patients use tobacco.[27] Worldwide, colorectal cancer is the third-most common cancer in men and second-most common cancer in women. These type of cancer cases are mostly reported from developed countries, whereas, in India most cases are reported from urban areas such as Thiruvananthapuram, Bangalore, Chennai and Mumbai.[28, 29] Gallbladder Cancer is rare but lethal and observed mostly in certain ethnic people from Chile, Japan, Poland, India, Pakistan and Israel. In India, most cases are reported from Uttar Pradesh, Bihar, West Bengal and Odisha, while in the North-East area, it is more common among woman.[30] Interestingly, it is observed that there is a reduced risk of developing breast cancer among women staying in rural areas in their early ages (up to 20 years)[31], the exact reason and relation between reduced breast cancer in women staying in rural areas are not well characterized. Hence, it requires more extensive study, highlighting the association, conditions and prevalence of cancer in different tribal populations. The awareness and monitoring of this disease should be a priority in endemic areas of tribal population.

All the above-mentioned diseases are linked with various factors, such as mutation in certain genes, development of immune response as well as change in a person's life style and socio-economic behaviour. However, the host immune response is the key factor that determines the level of disease susceptibility in various populations. For example, in endemic regions, it is observed that some individuals are more susceptible to malaria as compared to others. This varied susceptibility corresponds to the varied response of their immune system among different individuals. In depth, the genes responsible for the development of immune system are the one that decides the outcome of any infection. Single nucleotide polymorphism (SNP) in a gene of host immune system called as Toll like receptor 4 (TLR4) at Asp299Gly and Thr399Ile leads to susceptibility against malaria and tuberculosis.[32,33] In rural and tribal areas the study on such SNPs which are associated with certain disease types are required.

CONCLUSION

The health issues of tribal population living in endemic areas could be different from that of the urban population or people living in the rural areas due to various factors such as socio-economic status, life style and disease linked genetic mutations. This offers a major challenge for health programmes. But proper monitoring of certain diseases, which are most prominent in urban areas as well as those increasingly found in rural areas, need to be considered in tribal dominated regions. The study on hidden Trojan diseases in these endemic areas and tribal people hopes to open new vistas for situation analysis and disease detection.

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SHORT REVIEW

BLOOD GROUP GENOTYPING AND ITS RELATION WITH SEVERE *P. FALCIPARUM* MALARIA

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Abstract: *Severe malaria and its association to ABO blood group is reported among adults and children. The present review attempts collate such published reports to shed light on the conjecture and theory. All the studies support the hypothesis that individuals with O blood group are less prone to severe malaria despite no clear understanding of the mechanism of protection.*

INTRODUCTION

Malaria is a life threatening disease caused by *Plasmodium spp.*, transmitted through bite of infected Anopheles mosquito. Malaria is a huge global health problem prevalent in more than 100 countries. As per WHO estimates, India contributes about 75% of malaria cases in south-east Asia.[1] In India, 14 % of total malaria cases emanates from tribal population.[2] Of the five species of *Plasmodium* known to cause human malaria, *Plasmodium falciparum* is responsible for the most severe form. In the endemic areas, erythrocytes polymorphisms including ABO blood group also plays an important role in the clinical outcome of *P. falciparum* malaria along with other factors.[3, 4] This short review focuses on the role of ABO blood group in *P. falciaprum* malaria and its role in pathogenesis.

ABO Blood group

ABO blood group was discovered by Karl Landsteiner in 1900. ABO blood group refers to oligosaccharide antigen widely expressed on the Red Blood Cell (RBC) membrane, tissue cells, saliva and body fluids.[5] ABO blood group is coded by co-dominant alleles A and B located on chromosome 9 that express glycosyl-tranferase as well as O allele. Six main ABO phenotypes, O, A1, A2, B, A1B and A2B of human erythrocytes are known based on serological methods. The H antigen is present on all RBCs, excluding rare Bombay phenotype.[6] Individuals with A and B blood groups have A and B antigens respectively on their RBC surface while individuals with AB blood group have both A and B antigens together on their RBC surface.[4] Human ABO genes consist of 7 exons of varying size from 28 to 692 bp. A total of seven nucleotide substitutions (A297G, C526G, C657T, G703A, C796A, G803C, G930A) have been identified in A and B allele, of which 4 nucleotide substitutions (C526G, G703A, C796A and G803C) are non-synonymous substitutions.[5] These 4 nucleotide substitutions result in the change of amino acids R526G, G703S, L796M, and G803A respectively which

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lead to the different specificities for A and B glycosyltransferase. Six major genotypes of ABO blood group are known and a single base deletion observed in the O allele that renders glycosyl-transferase inactive.[7]

ABO blood group and severe malaria

Various studies in the past have shown association between ABO blood group and complicated or severe malaria from different geographical regions.[6] A study carried out by Fischer and Boone in Zimbabwe has shown that individuals with O blood group were less prone to severe malaria than non-O blood groups individuals.[8] Out of 53 symptomatic patients infected with *P. falciparum*, 27 patients with non-O blood group were also having less haemoglobin level than rest of the 26 O blood group patients (mean 11.7 g/dL, SD 2.6 versus 12.3 g/dL, SD 2.4; P = 0.36).[8] In Gabon, Africa, the association of blood group A with severe malaria was noted.[9] In a hospital base study from Sri Lanka, cases of *P. falciparum* severe malaria was significantly lower in O blood group patients (P=0.0003) than AB blood group patients (P<0.0001).[10] In another study from Ethiopia, cases of severe malaria were twice in A blood group [odds ratio (OR) 0.42, 95% confidence interval (CI) 0.20-0.88, P = 0.019], and more than two fold in B group [OR 0.38, 95% CI 0.16-0.89, P = 0.02] as compared to O blood group.[11] This correlation was not observed in case of severe malaria only but also with *P. falciparum* infection, where proportion of A or B blood group was significantly higher in *P. falciparum* infected individuals as compared with non-infected individuals.[12] All these studies have shown the association of non-O blood group with severe malaria while the prevalence of severity varies with different non-O blood group. Similar association was also seen in a case control family based association study wherein genotyping of >9000 individuals involving 3 African population, showed that A and B alleles were associated with greater risk for severe malaria than O allele.[13] The study also indicated that variations in the glycosyltransferase gene may also influence the disease severity in addition to blood groups.

A study among children of ages between 3 months to 12 years old in Ghana showed similar trend.[14] Wherein O blood group was less prone to severe malaria than non-O blood group.[14] In India, Panda et al (2011) has shown the less prevalence of severe *P. falciparum* malaria in O blood group individuals.[15] It has been investigated that protection against severe malaria in O blood group individuals is may be due to less formation of rosette.[16] Rosette formation is characterized by binding of *P. falciparum* infected RBCs to uninfected RBCs obstructing blood flow in the small blood vessels and leads to pathogenesis (Severe anaemia, cerebral malaria, metabolic acidosis) of *falciparum* malaria.[16]

Role of rosetting in malaria susceptibility

In vitro studies performed on clinical isolates of *P. falciparum* from patients with severe malaria have shown the formation of rosette.[17] The rosette formation was observed preferentially in erythrocytes of

A, B and AB blood groups than O group. Moreover, rosettes were also comparatively larger in these blood groups.[18,19] Rosettes formation has been demonstrated to cause obstruction to blood flow in rats.[20] Above studies support the hypothesis that protection of O blood group individuals from severe malaria may be due to lesser formation of rosettes. Further, A antigen has been shown to act as a co-receptor for rosetting in *P. falciparum* infection which further support that A blood group individuals may be more susceptible to severe malaria.[21]

CONCLUSION

All the previous studies have inferred that non-O blood group individuals are at higher risk for severe malaria. Further, it also supports the hypothesis that severe malaria results from rosetting of malaria parasite. However, very few studies have been carried out to ascertain the association of ABO blood group with asymptomatic malaria and placental malaria. Thus more studies are needed to explore the relationship between ABO blood and susceptiblity to severe malaria. It is worthwhile also to investigate potential of medicines and vaccines capable of disrupting the parasite rosetting to boost interventions against malaria.

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REVIEW

IMPORTANCE OF RAPID DIAGNOSTIC TEST IN DISEASE ENDEMIC AREAS

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Abstract: *Malaria is responsible for the high mortality in the majority of malaria endemic countries. Despite, several malaria control programs, it still causes many deaths. Accurate and timely diagnosis is a pre-requisite for reduction in complication and mortality. Traditional diagnostic methods remain challenging and require trained experts to interpret the results. WHO recommends the use of Rapid Diagnostic test (RDT) as a diagnostic tool in endemic areas where health facilities are under-equipped. But due to poor sensitivity and specificity with high number of false negative results, there is a need to further improve the quality of RDTs in a step towards malaria elimination especially from endemic regions.*

INTRODUCTION

Malaria presents a diagnostic challenge to laboratories and is associated with high burden of mortality and morbidity in many countries. Worldwide 4,38000 deaths have been reported and 3.2 billion people are at risk of infection.[1] Globally, South East Asian Region contributes to about 10% of all malaria cases. In India, around 11,26661 cases and 287 deaths were reported in 2015.[2] Delay and poor diagnosis contribute deaths due to malaria. Majority of malaria cases and deaths in India were reported from Madhya Pradesh, Chhattisgarh, Orissa, Gujarat, Jharkhand, Maharashtra, Assam, West Bengal and Karnataka particularly from the remote areas.[3] These remote areas have poor health infrastructure and inaccessible to dense forest.[4] People living in these remote areas often use symptoms like fever, chills, headache, muscle pains, nausea and vomiting to diagnose malaria but these symptoms often overlap with other tropical diseases and can be misleading and also encourage indiscriminate use of anti-malarial drugs.[5,6] Therefore, accurate and prompt diagnosis of malaria is essential requirement to reduce morbidity, mortality and transmission.

To overcome the non-specific clinical signs and symptoms, confirmatory laboratory diagnosis is essential. Microscopy is considered as a gold standard for detection of *Plasmodium* species. It also permits determination of sexual stage of the circulating parasite and density. However, it is laborious and requires skilled microscopist for its interpretation, which is often not available in remote and rural areas. Sometimes, parasite could be sequestered and are not present in peripheral blood of patients, thus parasite can be missed and cannot be easily

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diagnosed by the microscopist.[7] These limitations lead to poor interpretation of results and thereby causing therapeutic delay. Quantitative Buffy coat (QBC) is another method of diagnosis wherein parasite deoxyribonucleic acid (DNA) is stained using acridine orange fluorescent dyes and visualised under fluorescent microscope. Acridine orange is sensitive and can be used in epidemiological studies, particularly due to their high sensitivity even at low parasitaemia.[8] Although QBC is reliable, simple and user friendly, it requires cost intensive fluorescent microscope. Polymerase chain reaction (PCR) is another molecular method in which specific amplification of selected regions of malaria genome are used for diagnostic purposes.[9] It is highly specific and can detect low concentration of parasites (1-5parasite/µL of blood) and importantly, it can detect even mixed infections that are generally misdiagnosed by microscopy.[10] However, PCR is time consuming and not suitable to field settings due to requirement of specialized equipment, infrastructure and continuous power supply. Another molecular method of diagnosis is Loop mediated isothermal amplification (LAMP) which is considered simple and reliable for screening of malaria parasites.[11,12] It is easy and low in cost than PCR, however, reagents used in LAMP require cold storage often absent in remote locations.

Rapid diagnostic test

WHO has emphasized the urgent need for simple and cost effective method of malaria diagnosis to overcome the shortfalls of microscopy.[13] In this regard, Rapid Diagnostic Tests (RDT) can be an effective tool to substitute for microscopy in remote areas. In comparison to microscopy, RDTs are faster, simple to use and requires no specialised training or instruments and can be used as a epidemiological tool for rapid screening of malaria.[14,15] RDTs can detect 100 parasite/µL, equivalent to 0.002% parasitemia and can be spot tested under field setup in secluded areas.[16] Requiring only a drop of blood, these lateral flow immuno-chromatographic tests detect specific antigen produced by the malaria parasite.[17,18] Commonly used RDTs target *Plasmodium falciparum* Histidine rich protein 2 (*pf*HRP2) and two enzymes used in the glycolytic pathways of *Plasmodium* parasite, namely plasmodial lactate dehydrogenase (pLDH) and aldolase. HRP2 is the most common malaria antigen targeted and is specific for *P. falciparum.* pLDH enzymes are the other major group of targeted antigens. Monoclonal antibodies against pLDH are commercially available for the detection of *Plasmodium* spp. (pan-malaria), *P. falciparum* and *P. vivax*. *Plasmodium* aldolases are also pan-specific in their reaction and have been used in a combination test with HRP-2 to detect *P. vivax* as well as *P.falciparum* in blood.

Limitations of RDTs

RDTs contribute greatly to controlling malaria in resource deficient countries[14], despite, issues of sub-optimal sensitivity and reliability. In fact, genetic variation in the *Pf*HRP2 sequence seen in different geographical locations, has led to false negative results, impacting the performance and reliability of RDTs. First

prospective field study in 8 endemic states of India reported 2.4% and 1.8% of sample, that were positive by microscopy but tested negative with HRP2 based RDT lacking *pfhrp*2 and *pfhrp*3 gene respectively, which led to false negative result in RDT. A total 14 different amino acid repeats were identified from *pfhrp2* gene and 8 different amino acid repeats from *pfhrp3* gene[19], presence and absence of these repeats could affect the binding affinity of antibodies used in RDT for the parasite antigen. If Monoclonal Antibodies (MAbs) used in any *Pf*HRP2-based RDT recognize epitopes residing in repeats that are not universally present, the test will give false negative results.[20] As compare to *Pf*HRP2, pLDH and aldolase appear to be conserved.[20,21] However, a recent study on Iranian isolates showed nucleotide substitution in *Pv*LDH and *Pf*LDH.[22] In Indian isolates synonymous substitutions and non-synonymous substitutions were reported for the first time in 2014 in the *Pv*LDH genotypes.[23] Similar observation was also seen in *aldolase* genes from 18 *P. vivax* isolates from five different regions. A single synonymous SNP was observed in 3 of the 16 isolates. Two of these isolates were from China, while other isolate was from the Philippines.[24] However no such variation in *aldolase* gene has been reported in Indian isolates.

The specificity, sensitivity, number of false positives and false negatives and temperature tolerances of these tests vary considerably, exemplifying the difficulties and challenges faced due to the current RDTs.

CONCLUSION

Malaria diagnostic tests should be fast, easy to perform and interpret. Recommended and current gold standard used for routine laboratory diagnosis of malaria is microscopy involving smear preparation, staining which is labour intensive and time consuming. Most importantly, it needs trained experts for interpretation of the results. In the past few years, several other techniques have been developed but their costs are enormous and require infrastructure that hamper their implementation in resource limited settings.

In this regard, RDT offer the possibility of rapid non-microscopic method for malaria diagnosis. It is easy to perform and requires little training to interpret the results. However, poor sensitivity compared to microscopy hinders its reliability or utilization for malaria diagnosis especially in remote areas. Thus, there is an urgent need to further improve these RDTs for effective use.

FUTURE PROSPECTS

Current RDTs lack specificity and reliability and calls for its enhancement and modification to acquire improved and versatile diagnostic tools. These improved RDTs are required to carry sensitive and specific monoclonal antibodies targeting proteins universally expressed throughout the life cycle of *Plasmodium* and whose genes are highly conserved, offers a great potential of alternative targets. Several alternative malarial diagnostic targets have been

identified and explored. These include heat-shock protein 70 (Hsp70)[25], hypoxanthine phosphoribosyltransferase (pHPRT), phosphoglycerate mutase (pPGM) lactate dehydrogenase (pLDH) and fructose biphosphate aldolase (pFBPA) [26], glutamate rich protein (GLURP) dihydrofolate reductase-thymidylate synthase (DHFR-TS) and heme detoxification protein (HDP)[27] and glutamate dehydrogenase (GDH)[28]. Thus, it is hoped that next generation RDTs will help in circumventing the shortfalls of present day RDTs.

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PATHOGENESIS OF *P. FALCIPARUM* MALARIA: A SHORT REVIEW

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Abstract: *Plasmodium falciparum is responsible for causing the most severe form of malaria and its pathogenesis is still not well understood. Insight into its complex pathophysiology the virulence may be attributed to its ability to evade the human immune system by modifying infected host red blood cells for adherence to the vascular endothelium. Various other factors involve in this process includes adhesion of parasite to brain vasculature, rosetting, clumping and antigenic variation. This mini review highlights the allelic variation in relation to disease outcome, host immune status and genetic factors which can provide further insight into the parasite's virulence mechanisms.*

INTRODUCTION

Malaria is one of the most widespread infectious diseases found in humans and is a major global health problem. India contributes about 70% of cases reported from South East Asia region.[1] In India, *P. falciparum* contributes around 67% of all malaria cases.[2] Among five species of *Plasmodium* infecting humans, *P. falciparum* is the most virulent parasite responsible for the majority of cases and deaths in the world.[3] In Africa, *P. falciparum* malaria is responsible for majority of deaths in children between the ages of 1 to 4 years.[4] In contrast, in South America and South East Asia (particularly India) adult populations are at higher risk for severe malaria.[5] Malaria is a complex disease and the pathogenesis due to malaria is a result of interactions between host and parasite factors that influence the severity and outcome. Interaction of parasite with erythrocytes during its asexual stage in the blood leads to the onset of all the clinical symptoms of the disease.

Malaria and its Clinical manifestation

Different signs and symptoms of malaria depend on the diverse host – parasite interaction that affects erythrocytes and circulation of such infected blood cells leads damage to various organs of the body.[6] Clinical outcome of disease differs between children and adults, and symptoms can range from asymptomatic malaria to symptomatic with mild to severe affecting several tissues and organ. These symptoms range from high fever to multi-organ dysfunction, affecting brain (Cerebral Malaria [CM]), lungs (Acute respiratory Distress syndrome [ADRS]) and liver (Jaundice) and kidneys (Acute renal failure [ARF]). It can be fatal in case of metabolic acidosis and renal failure in adults which is less common in chidrens.[7,8] In general, CM, Severe Malaria Anemia (SMA) and Acidosis are common complications in case of children while ARDS and ARF are very rare.[9] In children complications can appear individually or overlap, wherein young children predominantly show

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severe malaria rather than CM as in case of older children.[10]

Scenario of severe malaria in Tribal area of India

In India, tribal population mostly resides in remote areas, limiting the access to outside world due to its typical geographical conditions. Sharma *et al* (2015) also reported that higher prevalence of malaria among tribal population[11], it could be due to various factors such as presence of various species of parasite and diversity of vector species, geographical and climatic conditions. These factors may be responsible for high transmission of malaria in tribal area.[12] Although very few studies were carried out to estimate the burden of severe malaria in the tribal population and reported various complications. In a study, burden of Cerebral Malaria was conducted in Madhya Pradesh and found that patients exhibited severe complications such as jaundice (26%), ARF (22%), ADRS (22%), SMA (18%), hypotension (17%), hepatic encephalopathy (7%), hypoglycemia (4%), and hematuria (5%). Seizure and SMA were higher in children whereas percentage of Jaundice, ARF and hematuria was elevated in adults and ARDS in CM patients.[13] Similar study in tribal population of Chhattisgarh was carried out and prevalence of *P. falciparum* complicated malaria (SMA, ARF, ADRS, Jaundice, Hypoglycemia and CM) was 13.5% among the *P. falciparum* malaria cases. Severe complications were majorly associated with children of age of 4–5 years and declined with increasing age. Cerebral malaria and severe malaria fatality rate due to *P. falciparum* was observed to be 32% and 9% respectively.[14] Odisha is also one of the tribal settlement states with high incidence of malaria. In Koraput, it was observed that children had a number of complications due to malaria severity. Major complications were hyperpyrexia (70.7%), CM (9.4%), SMA (7.7%), algid malaria (1.5%), and ADRS (2.2%), hepatitis (2.0%), urinary tract infection (1.8%), enteric fever (3.3%), and sickle cell disease (1.2%).[15]

Severity of *P. falciparum*

Pathogenicity of malaria is mainly due to parasite and host factor as ability of parasite to infect large number of RBCs (Red Blood Cells) affecting many tissues and organ of the body and second, due to involvement of host immune mechanism that induces the production of cytokines that contributes in severity of disease.[16]

Parasite Factors

P. falciparum parasite is known to infect all stages of RBC whereas other parasite species cause less severe form of malaria show preference to infect younger (reticulocytes) or older RBCs.[17] This preferential infection of RBCs also indicates the underlying cause of differences in clinical presentation as compared to other species of P*lasmodium*.[18] *P. falciparum* blood stage is short span (i.e. 48hrs) that gives it an advantage to multiply at a faster rate than other species. One of the unique characteristic possessed by *P. falciparum* is its ability to sequester or adhere to different cell/organs of the body (Heart, lung, brain, liver, kidney, subcutaneous tissues and placenta), thus, evading the splenic clearance and thus escaping the

immune response. A key feature of *P. falciparum* is cytoadherence (stick) to endothelial cells of blood vessels causing obstruction to blood flow.[6] Both cytoadherence and sequestration indirectly favours parasite multiplication.[19] Sequestered parasites do not circulate and are also unable to cross the inter-endothelial slits of the spleen. Another specific feature associated with severe malaria is formation of rosettes. It is phenomenon of spontaneous binding of parasitized erythrocytes to uninfected erythrocytes with mature asexual parasites.[20]

Antigenic Variation

The most novel survival mechanism of the parasite is antigenic variation. This is nothing but the changing of antigens on the infected erythrocytes surface. Virulence of *P. falciparum* is due to the expression by parasite-derived antigens on the surface of IEs, generally known as variant surface antigens (VSAs) and its strong affinity to adhere in vascular endothelial cells. Virulent proteins of *P. falciparum* include *P. falciparum* erythrocyte membrane protein 1 (PfEMP1)[21], repetitive interspersed family (RIFIN) proteins[22], sub-telomeric variable open reading frame (STEVOR) proteins[23], surface-associated interspersed gene family (SURFIN) proteins [24] and possibly others such as *P. falciparum* Maurer's cleft 2 transmembrane (PfMC-2TM) proteins.[25] Parasite-modified erythrocyte band 3 has also been proposed as a surface antigen or ligand for infected erythrocytes sequestration.[26] One of the key factors that provide sequestration on infected erythrocytes surface is the expressions of knob structures and the major structural component is knob-associated histidine-rich protein (KHARP). KHARP is found to have interaction with the erythrocytes cytoskeleton component such as spectrin and action that result in reduced membrane deformability.[27] Among the virulent parasite derived proteins major virulence factor is PfEMP1 presented by knobs on external surface of infected erythrocytes membrane. Some polymorphic surface antigens which are involved in chronicity of disease are merozoite surface protein (MSP) family, apical membrane antigen 1 (AMA1) and erythrocyte binding antigen-175 (EBA-175). *P. falciparum* MSP family plays a crucial role in the parasite's invasion and manifestation of severe malaria. A study conducted by Sahu *et al*., (2008) in Odisha, found that RO33 subtype of *msp-1* and the 3D7 subtype of *msp-2* were associated with CM and SMA.[28] The study carried out in Bastar district of Chhattisgarh state, showed K1 allelic family of *msp-1* and IC1 family of *msp-2* were directly associated with malaria severity (Sneha *et al* unpublished data).

Host Factors

Various host receptor involved in adhesion to parasite ligands that mediates cytoadhesion and sequestration are intercellular adhesion molecule 1 (ICAM-1, CD54), platelet/endothelial cell adhesion molecule1 (PECAM-1, CD31), vascular cell adhesion molecule1 (VCAM-1), thrombospondin, E-selectin, Pselectin, CD36, and chondroitin sulfate A.[29] Association of ICAM-1 binding to endothelial cells in severe malaria has been observed in CM or SMA patients as

compared to patients with uncomplicated malaria.[30] Most parasite ligands (antigens) majorly binds to CD36 adhesion molecules, and it is considered as an important receptor for sequestration of infected erythrocytes.[31] The mechanism of rosetting is mediated through binding of various host receptors that are present on uninfected erythrocyte such as complement receptor 1 (CR1), blood group antigens A and B, glucosaminoglycans and Heparin Sulphate (HS) proteoglycans.[32]

Immune Response and Pathogenesis

Immunological mechanisms involved in pathogenesis of malaria, through production of pro-inflammatory cytokines. Proinflammatory cytokines: IL-1, IL-6, IL-12, IFN-gamma and TNF-alpha have been known to play important role in protection against malaria in *Plasmodium* infection. But the over production of these cytokines which results due to genetic variation of host have been reported in severe malaria cases leading to tissues and organ damage.[33] TNF-alpha has been proposed to induce expression of ICAM-1 in brain endothelial cells in CM cases [7]. TNF alpha, IL-3 and granulocyte colony stimulating factor (GM-CSF) are responsible for causing the neurological symptoms while elevated levels of IFN-gamma, chemokine, CXCL10, plasma CXCL10 and CXCL4 are associated with mortality of cerebral malaria.[34, 35]

Perspective

Pathogenesis of malaria is a complex process which involves various clinical conditions that have distinguishable features. For the proper treatment and prevention of malaria it is very crucial to study various host parasite factor that contribute to severity of disease. As large numbers of host and parasite factors have catastrophic effect on disease outcome. Such factors can be targeted for developing new tools for the intervention program. Detailed knowledge of host genetics and parasite will help to provide an insight in the persistence of this disease. Further the research work needs to understand to what extend parasite adhesion or endothelial phenotypes and antigenic variations may contribute to the malaria pathogenesis.

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COMPARATIVE MODELING AND INSILICO ANALYSIS OF *PLASMODIUM VIVAX* VON WILLEBRANDFACTOR A DOMAIN-RELATED PROTEIN

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Abstract: *Malaria is one of the most serious diseases caused by protozoan parasite of genus Plasmodium. The malaria parasite has a complicated life cycle, and efforts are being made to develop anti-parasitic vaccine against each stage. Transmission-blocking vaccines are one strategy for controlling malaria, whereby sexual-stage parasites are inhibited from infecting mosquitoes by human antibodies. In the present study, we have focused on malaria transmission-blocking vaccine candidate named Plasmodium vivax von Willebrand factor-A domain-related protein (PvWARP). PvWARP plays an essential role in ookinete-to-oocyst development during malaria parasite development. Since, the crystal structure of PvWARP is not yet available in the protein data bank (PDB), to deduce its structural and functional information, a three-dimensional protein structure was constructed using protein structure prediction server - phyre2.Several quality assessment and validation parameters were computed which indicated that the designed homology model is reliable. Validated structure was successfully submitted to the online protein model database and was assigned PMDB ID: PM0079777. Furthermore, modeled PvWARP 3D structure can be used as a reference in several different computational approaches like insilico antigen-antibody docking or protein-protein docking studies.We expectthe predicted PvWARP homology model will guide researchers in designing transmission-blocking vaccine against malaria in the absence of experimental structural data.*

Keywords: Comparative modeling, 3D Structure, Malaria transmission-blocking vaccines, PvWARP

INTRODUCTION

Malaria is a mosquito-borne,life-threatening ancient parasitic disease caused by parasite of the genus *Plasmodium.* The disease significantly impacts global health; with 135–287 million cases and 627,000 deaths annually.[1] Life cycle of the malaria parasite alternates between the human host and the anopheles mosquito. Among the 5human malaria species, *Plasmodium falciparum* is the most severe form, causing malignant malaria globally, while in areas outside the African continent, *P. vivax* is responsible for more than 50% of all malaria cases, yet the morbidity associated with this infection and its spectrum of disease is less studied.[2] In the past, the disease caused by *P. vivax* was considered benign as

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compared to *P. falciparum.* However, recent reports indicate that, similar to *P. falciparum*, this parasite can also cause complications and thus increases severity of the disease.[3,4] There is emergence of drug resistance in *P. vivax.*[5] Therefore, the development of new control strategies such as a safe and an effective vaccine is expected to play an important role in controlling deadly disease. Research on novel *P. vivax* drug and vaccine targets currently lags far behind that of *P. falciparum*, partly due to the difficulty of establishing a long-term in-vitro culture. To date only two P. vivax candidate vaccines have reached phase I clinical trials, as compared to 23 P. falciparum vaccine candidates, several of which have progressed to Phase II and III clinical trials.[6]

Malaria vaccines are classified into three types based on the parasite life cycle target stage: Pre-erythrocytic vaccines, blood stage vaccines and transmission-blocking vaccines (TBVs).[7] Liver stage vaccines will reduce the chances of a person becoming infected. Asexual blood stage vaccines will reduce disease severity and risk of death during infection. A transmission blocking vaccine will target the sexual stage of the parasite and prevent the spread of malaria through the community; such a vaccine would have the potential to reduce the burden of disease and death from malaria. The aim of this vaccine is to block transmission of malaria from mosquitoes to humans by preventing the malaria parasite from developing within the mosquito. The inclusion of a transmission blocking vaccine would also greatly prolong the useful life of vaccines against other stages by preventing the spread of parasites that become resistant to these vaccines.

The Ookinete-secreted protein named, *Plasmodium vivax* von willebrand factor A domain-related protein (PvWARP),is a promising candidate for malaria transmission-blocking vaccines.[8,9] TBVs are based upon antigens expressed on the surface of the sexual and mosquito mid-gut stages, such as gametocytes, gametes, zygotes and ookinetes form of malaria parasite.[10-12] These antigens are the targets of antibodies induced by vaccination of the host. A TBV candidate, PvWARP is expressed in late ookinetes and early oocysts stage.[13] It plays an important role in oocyst development inside the mosquito.[13,14] Experiments show that, oocyst formation was reduced significantly when mosquitoes fed on an infected mouse passively immunized with the anti-PvWARP antibody[8]. This indicates that the antibody interferes with PvWARP function by recognizing the protein on the surface of the parasite and makes it an important candidate antigen for a TBV.

The 3D structure of PvWARP is not known experimentally yet, therefore computational method was focused to build a good quality model through comparative modeling approach. Protein structure determination using experimental methods such as X-ray crystallography, NMR spectroscopy and electron microscopy are expensive, time consuming and often difficult processes.[15] Due to which there has been a huge gap

of information between DNA/protein sequence information and experimentally derived 3D structures in database. Computational modeling techniques are good alternatives to overcome these problems in protein structure prediction with higher resolution and accuracy in considerably less time.[16,17] Homology or comparative protein modeling is one of the computational method for 3D structure prediction using closely as well as distantly related experimentally determined protein structures as templates.[18] Understanding the mechanism of protein function generally requires knowledge of protein three-dimensional structure, which is ultimately determined by protein sequence, allowing us to study its various structural and functional features. The present study aims at prediction of PvWARP 3D structure followed by evaluation of the predicted 3D structure.

MATERIALS AND METHODS

Retrieval of Target Sequence and Physiochemical characterization

The Amino acid sequence of PvWARP was retrieved from the national center of biotechnology information (NCBI), in fasta format, with the accession number BAB55890.1. Expasy's protparam proteomics server[19] was used to predict physiochemical properties such as molecular weight, theoretical pI, total number of negatively (Asp+Glu) and positively (Arg+Lys) charged residues, extinction coefficient, instability index and grand average of hydropathicity (GRAVY).

Prediction of 3-D Structure via Comparative Homology Modeling

The protein sequence was subjected to comparative homology modeling via phyre2 Server.[20] It is a major update to the original phyre server with a wide variety of new features; accuracy is improved, using the alignment of hidden markov models via HH search to significantly improve accuracy of alignment and detection rate.

Energy Minimization, Quality Assessment and Visualization of Predicted Structure

The model constructed was subjected to energy minimization using high resolution protein structure refinement server –modrefiner.[21] Once the 3D model was generated, structural evaluation and stereo chemical analysis were performed using prosa-web[22] z-scores and procheck Ramachandran plots.[23] Visualization of generated model was performed using UCSF chimera 1.5.3 workbench[24] and swisspdb viewer.[25]

Characterization of the Modeled Structure

Secondary structure prediction and characterization of protein at functional level were done using profunc server.[26]

Profunc identify the likely biochemical function of a protein from its three-dimensional structure. It uses a series of methods, including fold matching, residue conservation, surface cleft analysis, and functional 3D templates, to identify both the protein's likely active site and possible homologues in the PDB.

RESULTS AND DISCUSSION

Based on expasy's protparam tool predictions (Table 1), the vonwillebrand factor A-domain-related protein (PvWARP) contains 300 amino acids, with a predicted molecular weight of 33208.6 Daltons and an isoelectric point of 6.09. An isoelectric point below 7 indicates a negatively charged protein corresponds to having more negatively charged residues and an instability index of 34.49 suggesting a stable protein. The negative GRAVY index of -0.331 is indicative of a hydrophilic and soluble protein. Secondary structure predictions revealed that protein has 5 sheets,2 beta alpha beta units,4 beta hairpins,1 beta bulges,15 strands,7 helices,3 helix-helix interacs ,40 beta turns and 9 gamma turns. (Figure1).

Table 1. Physiochemical Parameters computed using Expasy's ProtParam tool

S.No.	*Physiochemical Parameter*	Value
1	*Number of amino acids*	*300*
2	*Molecular weight*	*33208.6*
3	*Theoretical pI*	*6.09*
4	*Total number of negatively charged Residues (Asp+Glu)*	*38*
5	*Total number of positively charged Residues (Arg+Lys)*	*35*
6	*Extinction coefficient*	*46005*
7	*Instability index*	*34.49*
8	*GRAVY*	*-0.331*

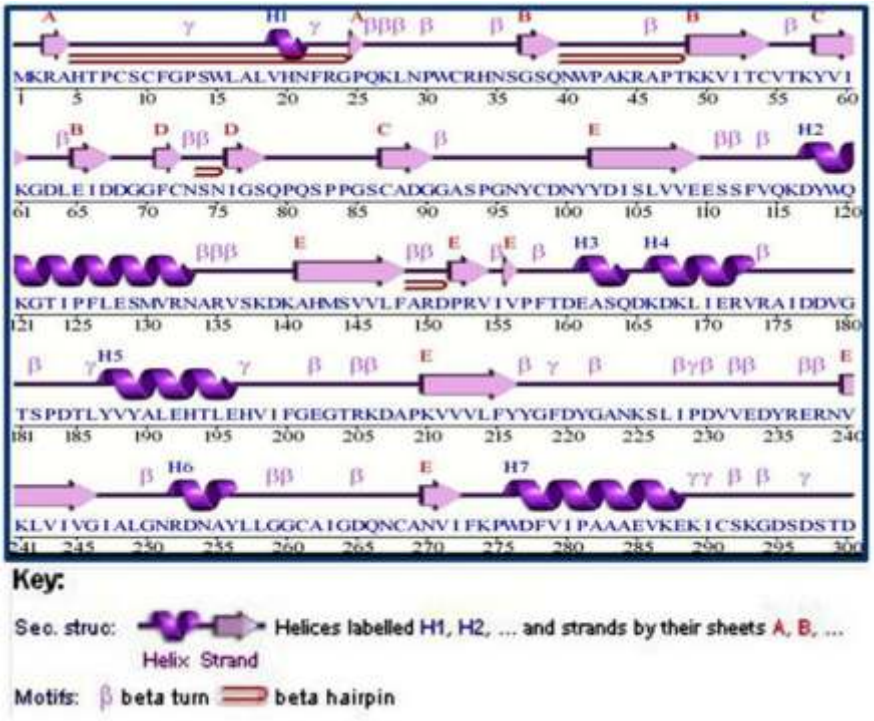

Figure 1 : Secondary structure prediction using Profunc Server

Homology Modeling, Structure Validation and Functional Annotation of PvWARP

Modeled 3D structure generated by phyre2 server was subjected to energy minimization and assessed for both geometric and energy aspects. Several structure assessment methods including, Z-scores, and procheck Ramachandran plots were used to check reliability of the final PvWARP 3D model (Figure 2). Procheck displayed 92.6% of residues in the most favored regions, with 7.0%, 0.4% and 0.0%residues in additionally allowed, generously allowed and disallowed regions, respectively (Figure 3). None of the residues was present in the disallowed region of the Ramachandran plot. This indicated that the backbone dihedral angles, phi and psi, in the PvWARP3D model, were reasonably accurate. Good quality models are expected to have more than 90% of amino acid residues in most favored and additional allowed regions.

The quality of the present model is evaluated as good and reliable. The Z score is indicative of overall model quality and is used to check whether the input structure is within the range of scores typically found for native proteins of similar size. Z-scores of the PvWARP model is -5.22 (Figure 4). The score is well within the range found for proteins of similar size indicating a highly reliable structure. The profunc result analysis suggests that the protein is involved in process of cell adhesion with the presence of one major domain named von willebrand factor (vWF) type A domain belonging to vWA-like superfamily (Figure 5).

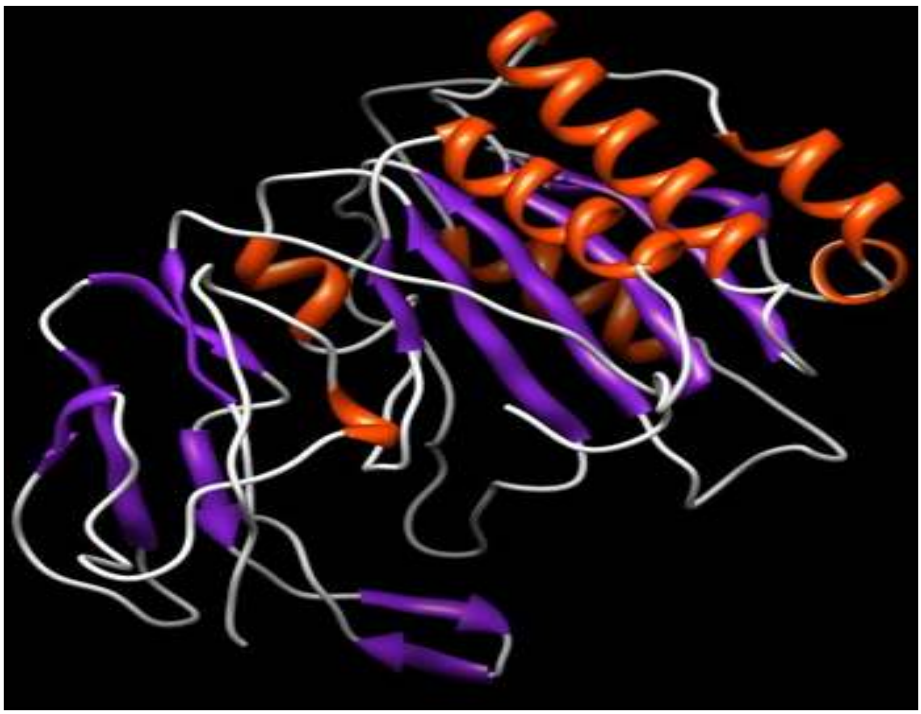

Figure 2: Predicted PvWARP 3D model. Alpha helices are shown in orange, beta sheets in purple and coils in grey colour

Accession number of Protein structure

The predicted 3D structure of *Plasmodium vivax* von Willebrand factor A-domain-related protein (PvWARP) was successfully deposited to the protein model database (PMDB).[27] Submitted modeled structure was assigned a specific accession number. This accession number can be used to retrieve the submitted protein structure PMDB ID: PM0079777 (Figure 6).

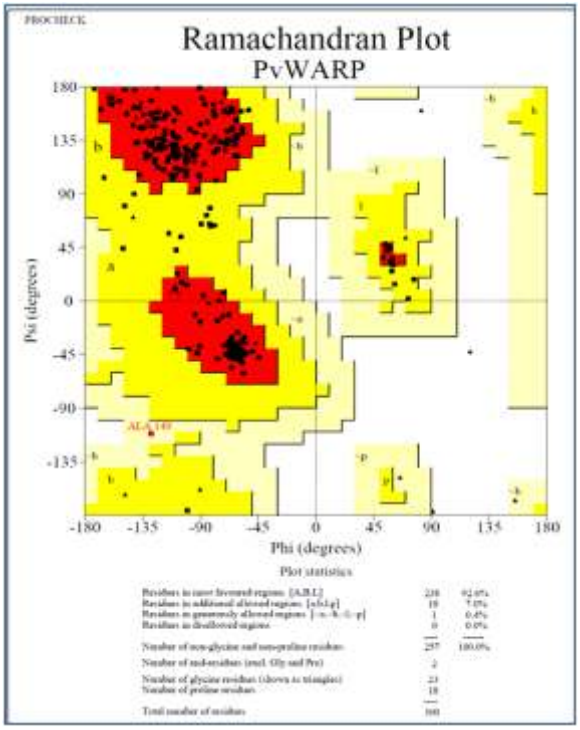

Figure 3: Ramachandran Plot generated using Procheck

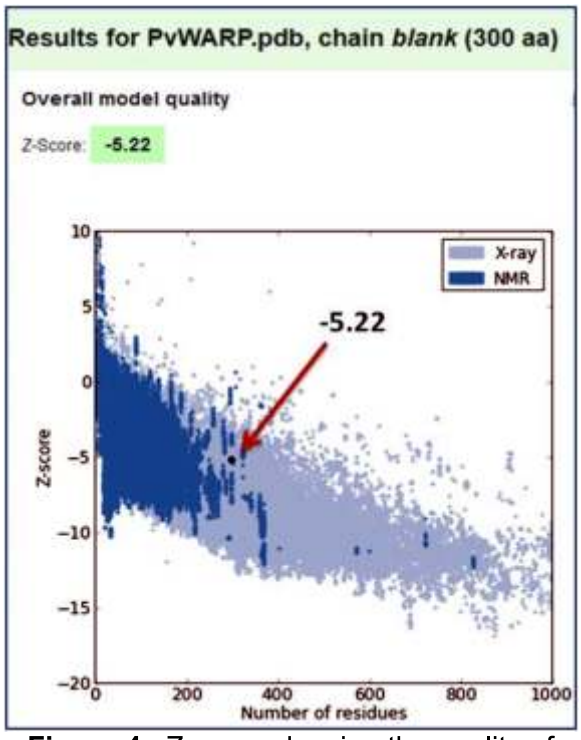

Figure 4 : Z score showing the quality of 3D structure

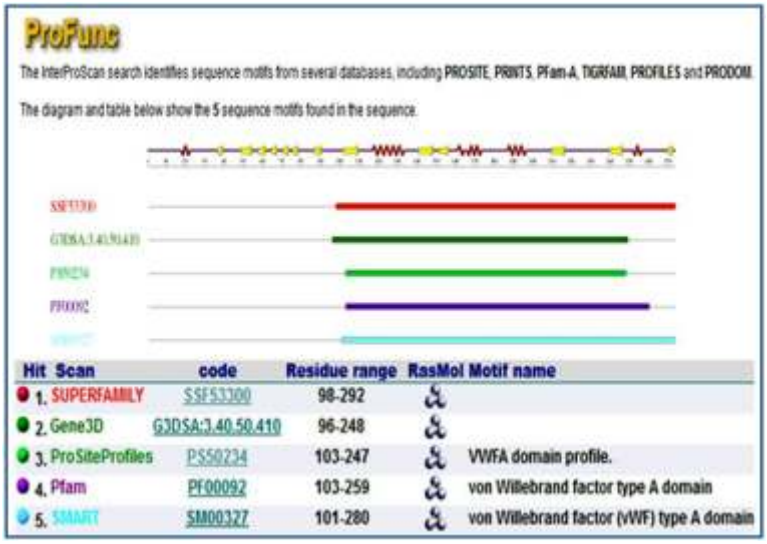

Figure 5 : Profunc result shows predicted conserved domain in the protein sequence

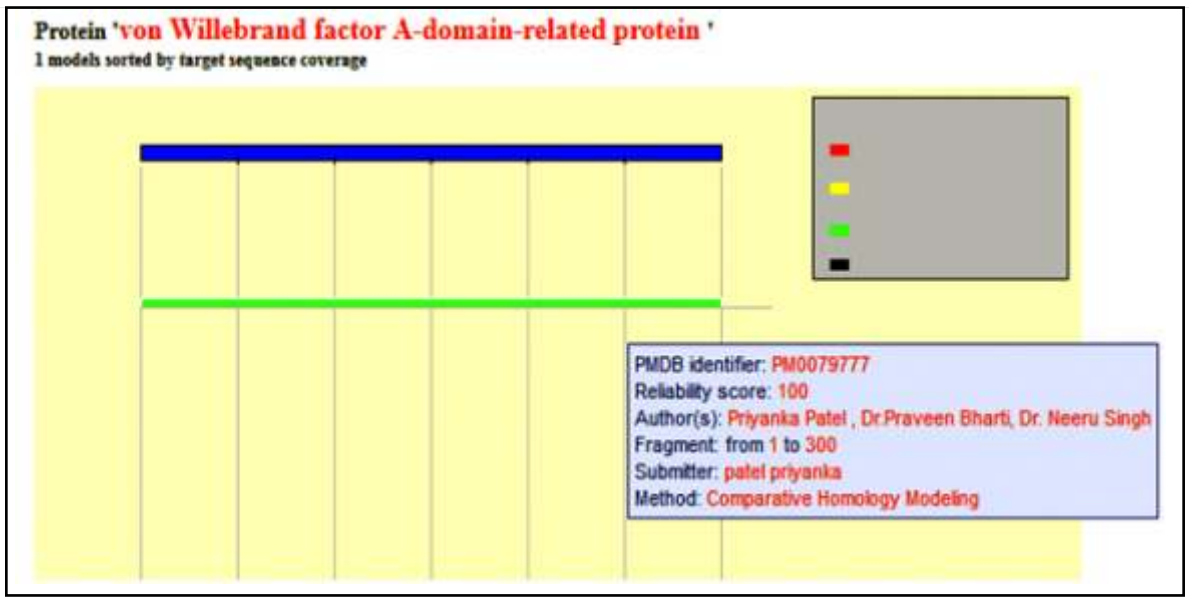

Figure 6: Screenshot of PMDB database showing submitted PvWARP model with its Accession Number

CONCLUSION

Knowledge of the detailed structure of a protein is crucial to our understanding of the biological functions, interactions, antigenic behavior, and predicting novel functions. Modeled PvWARP 3D structure can be further used in insilico studies like computational antibody-antigen docking or protein-protein docking to understand the nature of the antigen-antibody interactions in greater details, that would provide new insights into mechanism of transmission blocking and may helpin understanding the biology of the malaria parasite inside mosquito midgut. This form of computational analysis may be utilized as instrument for the future development of malaria vaccines.

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WAY TO MALARIA CONTROL: RESISTANCE IN *PLASMODIUM FALCIPARUM*, CURRENT STRATEGIES AND FUTURE DIRECTION

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Abstract: *Drug therapy remains the mainstay of malaria control and elimination. Malaria control is compromised by the spread of Plasmodium falciparum resistant strains and has led to a significant resurgence of malaria morbidity and mortality. P. falciparum has developed resistance to drugs commonly given to cure malaria has became one of the major obstacles in malaria intervention. The present article focuses on the drawbacks of successful implementation of antimalarial agents to control and/or possibly eradicate this dreaded disease.*

BACKGROUND

Malaria is rampant in India, and one predominant reason is the prevalence of vast tribal population.[1,2] In Madhya Pradesh, the central state of India, malaria in tribal region is mainly caused by the two most common species namely, *P. falciparum* and *P. vivax* with the preponderance of *P. falciparum.* Complications in human hosts are caused by both the species but *P. falciparum* is more severe and fatal.[3,4] For the eradication of malaria, the twofold course of action is generally applied: (i) malaria vector control, and (ii) effective drug treatment.[5] But the treatment and control is greatly hampered due to the rapid increase of drug resistant species. The cases of *P. falciparum* type malaria are ascending because of the development of resistance in them against majority of common antimalarial drugs.[6] Long before independence there was no organized program for malaria control. Later, serious efforts were made in 1953, and the first organized National Malaria Control Program came into existence. During the period between 1950s-1970s extensive efforts were made for the reduction of transmission of this disease by vector control, indoor residual spray, surveillance for active case detection and treatment.[7] India reported its first case of *P. falciparum* showing resistance from wonder drug, Choloroquine in 1973 from the state of Assam.[8] Soon after the failure of Chloroquine, Sulphadoxine-Pyrimethamine (SP) was used to treat uncomplicated choloroquine resistant *P. falciparum* malaria cases but later SP also was rendered ineffective. It was noted that the parasite had developed resistance against SP in the year of its implementation (1979) in Assam, propagating even more rapidly to other parts of the country.[9] Despite vigorous attempts to control malaria, all the strategies and approaches failed in the wake of drug resistance.

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WISH for malaria control

Weaknesses - To prevent complications in any disease, and to save lives, prompt diagnosis followed by proper treatment is the recommended protocol. The complexity of malaria control has increased manifolds in the vast tribal areas of Madhya Pradesh (15% of India's tribal population) because of its difficult terrain such as deep valleys, hills and hillocks covered with dense forest.[4,10] Baiga tribe known as the most vulnerable tribes contributes 12-15% of malaria cases in the state.[6] They belong to the aboriginal group with low levels of education, poor socio-cultural development, and invariably lead an isolated life. They don't utilize modern medical facilities available to them, but believe in spiritual healing of diseases as first line of treatment, leading to underutilization of health services provided to them.[11] Other factors such as environmental factors, malnutrition, lack of proper diagnosis, inadequacy of expert scientific personnel, no electricity and lack of knowledge also contribute significantly. Also, climatic conditions favours the growth of various malarial parasites and vector species which results in high malaria transmission in these areas. Owing to difficult terrain, access to such areas is limited which makes it difficult to establish health care infrastructure thus hindering the successful implementation of malaria control program. Inadequate supply of antimalarial drugs and spray coverage are the secondary major issues causing increase in malaria mortality and morbidity.[12] Further, the units providing health services are often understaffed, and also the constant movement of people in and out of the forest makes the diagnosis and treatment difficult.[13]

Impede – Malaria is preventable and treatable disease if diagnosed early. Malaria burden can be monitored in endemic regions by the rigorous use of Rapid Diagnostics Tests (RDTs) and it also reduces the dependency on traditional microscopy.[14,15] But, there are few limitations of RDT- technical concern at high temperature, improper detection of low density parasitaemia and false positive results. However, the most practical way for mass screening currently is by RDTs. Hence, only good quality RDTs should be used for optimal results[16] which can further reduce the emergence of antimalarial resistance in such areas by precise diagnosis. Overcoming the scarcity of efficient RDTs needs to be tackled. The development of therapeutic antimalarial drugs is the main strategy of malaria control,[17] as no effective vaccine is available yet. Today resistance has emerged to virtually all antimalarials, therefore WHO has recommended the use of Artemisinin based combination therapy (ACTs) for the treatment of uncomplicated *P. falciparum* malaria.[6] Although the states have adopted ACTs in tribal districts, yet the supply is limited and only a small fraction of the population receives them. The rapid spreading of SP resistance parasites may decrease the present high efficacy of ACT in India, and therefore necessitate switching to a different kind of combination therapy. Unfortunately, recent emergence of Artemisinin

resistant parasites in Southeast Asia and near the Indian border is an alarming situation which could hamper the current elimination efforts.[18-20] Multidrug resistance and Counterfeit antimalarials are another threat to patient's health and antimalarial drug resistance in India. Fortunately, as of now, very few cases of multidrug resistant *P. falciparum* malaria have been reported; first case was from Kamrup district of Assam.[21] It is important to note that even mosquitoes have developed resistance against commonly used sprays like DDT, malathion and pyrethroids which were effective against vector control previously.[22,23]

Strategies – Recent technology like Rapid Diagnostic Test (RDT) kits made available to health workers and general public have made malaria control and elimination a real possibility. The effective ways to lower the pressure of malaria in these areas are the implementation of RDTs to remote and inaccessible parts, use of ACTs where cholorquine and SP have shown resistance, gametocidal drugs should be used in high transmission areas, Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) for vector control. Community participation, knowledge, awareness, attitude, behavior and interaction with local people play an important role in successful implementation of malaria control program.[24] In the long run, improvement in the socio-economic status of the community will have remarkable impact too.[25] To avoid emergence and spread of drug resistance parasites, continuous surveillance and monitoring is needed. Antimalarial drug resistance against ACT in *P. falciparum* can be detected in three way- therapeutic efficacy (*in vivo*), culture of parasite (*in vitro*) and molecular markers. Molecular monitoring offers the earliest way to detect emerging drug resistance and provide early warning.[6] All the above factors put together will greatly contribute towards eradication of malaria, reinforced with better policies and decisions about the usage of drugs and control of resistance.

Hope – New combination therapies are on the way using Arterolane as a substitute of plant derived Artemisinin. Arterolane is a synthetic analogue of Artemisinin and has the potential to replace plant-derived Artemisinin.[26] Trials are underway for combinations of current ACTs like Artesunate + Lumefantrine Artesunate + Piperaquine, etc. Advance molecular based technology like LAMP (Loop Mediated Isothermal Amplification) should be implemented at field level for accurate diagnosis.[27] With the help of community health workers, like ASHA, proper case management of malaria can be done at village levels.[24] For effective therapy and to reduce drug pressure, extensive study on parasite biology is needed.

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LEVEL OF KNOWLEDGE ON MATERNAL HEALTH CARE AMONG BAIGA TRIBE OF MADHYA PRADESH

A.K. Goel [a] and D. Kumar [b]

Abstract: *Complications during pregnancy and childbirth are still a leading cause of maternal morbidity and mortality among tribal women of Madhya Pradesh. Knowledge of Antenatal care services can ensure safe motherhood by reducing the delay in seeking care, visiting the health facility and receiving care. The objective of the study is to assess knowledge of maternal health care services among tribal women in Dindori, District of Madhya Pradesh. A cross sectional study was conducted in the Year of 2010. For the purpose 460 currently married women were interviewed using interview schedule in 24 villages. Respondents were selected using probability proportion sampling method. Data entry and analysis was done using SPSS version 20.* The results showed that *about 70% respondents were aware of antenatal care. Only 17%* women knew about first ANC checkup in first trimester of *pregnancy being essential. About 50% women had knowledge of safe delivery in health Institutions. Majority of respondents considered delivery conducted by untrained Dai was safe. Poverty, illiteracy and ignorance seem to be the main cause of concern among rural and tribal women. Awareness needs to be created in these areas regarding the antenatal care services and about the importance of Institutional deliveries.*

Keywords: Tribal, Maternal health, Antenatal care, Awareness.

INTRODUCTION

Maternal and infant mortality are indicators that show status of health care delivery systems. The maternal care includes care during pregnancy, and needs to begin at the early stages of pregnancy. The maternal mortality is a global burden, wherein about 287000 women were reported to have died due to pregnancy and child birth related complications in 2010.[1] These maternal deaths can be avoided if the preventive measures are taken on time. These include antenatal care to all mothers, delivery by skilled birth attendant and timely referral to hospitals etc. In rural and tribal areas most of the deliveries occur at home, far from emergency obstetric services or without access to skilled attendant. This is associated with increased risk of mother and child survival.[2,3] Knowledge of maternal and child health are considered essential for the health of both the mother and the child. For improving maternal, newborn and child survival among tribal women, imparting knowledge about maternal health care services and practices are much important, particularly in 'BIMARU' states, as it expected to reduce the MMR and IMR. In general, rural and tribal women of Madhya Pradesh are socially backward with low economic development, and illiterate with no awareness about the utilization of health services.[4,5] The reduction of mortality of

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women is a matter of concern for the governments across the globe. The Millennium Development Goals (MDG) of the United Nations[6] has set the target of achieving 200 maternal deaths per lakh live births by 2007 and 109 per lakh live births by 2015. India is likely to miss this MDG as one maternal death is reported every 10 minutes in the country and MMR has reduced from 212 in 2009 (SRS 2007-2009) to 200 in 2010. Moreover, over 56000 women died from pregnancy related complications in 2010.[7] To reduce the maternal and infant mortality, the Govt. of India launched CSSM program with assistance of World Bank & UNICEF. National policy 2000 and National health policy 2003 adopted by GOI strongly reiterated the government's commitment to reduce maternal and infant mortality by setting goals to reduce maternal mortality to 100 per lakh live birth and infant mortality 30 per live birth.[8,9] Reports indicate that IMR declined from 72 to 61 in rural area from 2001 to 2007 and MMR 327 to 301 from 2001 to 2003.[10] While IMR in rural area of MP is 77 compared to national figure (61) and MMR in MP is 269 compared to 212 in the country. In view of this Govt. of MP has introduced Janani Suraksha Yojana (JSY) under National rural health Mission (NRHM) for promoting institutional deliveries, full antenatal care (ANC) and postnatal checkup through ASHA (Accredited Social Health Activist) worker.

***The Tribe**:* The Baiga tribe is one of the most ancient and primitive aboriginal tribe of Madhya Pradesh and notified as particularly vulnerable tribal group (PVTG).[11] The habitat area of primitive Baiga's is known as Baiga-Chak of district Dindori of Madhya Pradesh.[12] They are confined to hilly and dense forest areas, isolated from the main stream, their livelihood mainly depends on agricultural pursuits and collection of forest produce (MFP).[13-15] They are socially and economically backward, illiterate and unaware of health care services. The main habitat areas of these tribes are 3 blocks of Dindori district, namely Bajag, Karanjia and Samanapur. The main purpose of this study is to identify the knowledge of maternal health care services among Baiga women in Madhya Pradesh.

MATERIAL AND METHOD

A study was carried out among Baiga tribe by National Institute for research in Tribal health (NIRTH), Jabalpur in 3 blocks Bajag, Samanapur and Karanjia in Dindori district of Madhya Pradesh in 2010. Majority of inhabitants of Dindori district (95%) lives in rural areas. Among these, 65.3% of total population are tribals. Significant portion of these tribals (37%) live below poverty line with poor health and education facilities.[16] A cross sectional survey was undertaken in 24 villages with 460 households to cover the desired sample of 500 ever married women arrived through probability proportion to size (PPS) sampling procedure. Out of 500 ever married women, 460 women (currently married women) responded to the study on knowledge on the issues. The information was recorded on the basis of detail interview after obtaining written consent. The level of knowledge about what needs to be done when pregnant, perception

about health problems that could occur during pregnancy, their initiative in utilization of antenatal care services, and decision about place of delivery and post natal care etc were ascertained.

RESULT AND DISCUSSION

Background of Respondent

Most of the respondents were illiterate (84.5%), dependent on labour including agricultural work (87%) and belonged to nuclear families (74%). About 96 % had no toilet facility in their homes and only 26% houses were electrified. The socio-economic condition of the tribe was poor.

Knowledge of Antenatal Care

Antenatal care (ANC) refers to pregnancy-related health care provided by doctor or health worker in a health institution or at home, ideally ANC monitor pregnancy for signs of complications, detect and treat pre-existing and concurrent problems of pregnancy, advice and counsel on preventive delivery cares, diet, post natal care and related issues. A pregnant woman can have an ANC after registering their name by visiting a doctor or another health professional. During the survey a question about who registered the name of pregnant women for ANC checkup was obtained from women in the reproductive age group for assessing their knowledge. The Table 1, depicts that 51% women were aware of Aganwadi registering for names of pregnant women for ANC checkup (X^2=184.31 at 3 degree of freedom), followed by ANM/LHV (24.7%), other health professional (13.5%) and don't know (11.0%). It was found that obstetric examination done was not satisfactory in majority of the cases. It was observed that Antennal mother received poor quality of services from ANM and no investigations during pregnancy period were performed.[15] In the context of antenatal services, 69% knew that at least one antenatal checkup needs to be done during pregnancy. Among them only 17% knew about need for first ANC checkup in first trimester (within 3 month), 29.3% in second trimester (4-6 month) and 22.6% in the third trimester (7-9 month). Most importantly, 31% were totally unaware of antenatal checkup periodicity. Interestingly, 72% respondents knew about need for at least one injection of tetanus toxid (T.T) but were totally ignorant about the name of injection and its benefits. The iron deficiency anemia is the most common micronutrient deficiency among pregnant women. All pregnant women must take supplementary Iron in the form of IFA tablets. Fifty nine percent Baiga women were aware of need for consumption of Iron tablet.

Table 1. Knowledge of Antenatal care (ANC) component

Component of ANC	N= 460	Statistical test
Knowledge of ANC		
yes	68.9%	
No	31.1%	
Month of first ANC check up		
First trimester	17.0%	Z=4.36* at P<.05
Second trimester	29.3%	significant between 1st and 2nd trimester
Third trimester	22.6%	Z=2.134* at P<.05 significant between 1st and 3nd trimester
Don't know	31.1%	
Who registered name of pregnant women		
Aganwadi	50.8%	X^2 =184.31*
ANM/LHV	24.7	significant at 3 d.f.
Other health professional	13.5	
Don't know	11%	
Knowledge of Tetanus toxoid-Injection		
Yes	72%	
No	28%	
Knowledge of Iron folic acid		
Yes	59%	
No	41%	

Care during Delivery

Knowledge for better place of delivery: Another important thrust of the reproductive and child health program is to encourage deliveries under proper hygienic condition and under the supervision of trained health professionals. Interviews included questions on the place of safe delivery and person assisting delivery. The Figure 1 shows that 49% of the women informed that births should take place in health institutions. About 48% indicated homes, 2% in other places and 1% in relative's home or parental home.

Knowledge of assistance during delivery: Most of deliveries which take place at homes are assisted by medically untrained persons, which lead to higher maternal morbidities and mortality.[14,15] For safe delivery, women should be informed that trained personnel need to be sought during delivery. The Figure 2 shows the response on assisted delivery. Majority of women (36%) indicated that untrained Dais can assist during the delivery followed by ANM (33%), Trained Dai (12%), Doctor (9%), traditional birth attendant (3%) and Gunia/Healer (3%).

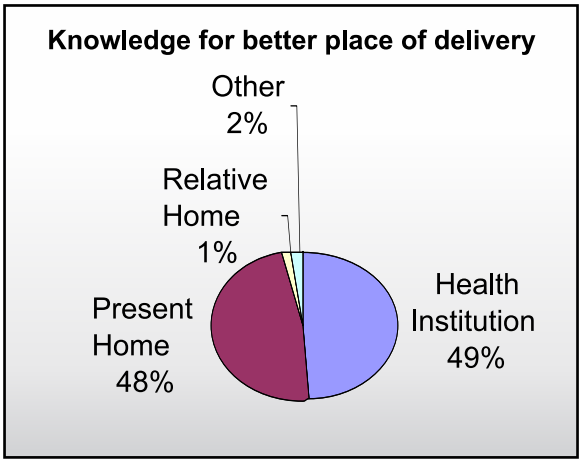

Figure 1: Knowledge for better place of delivery

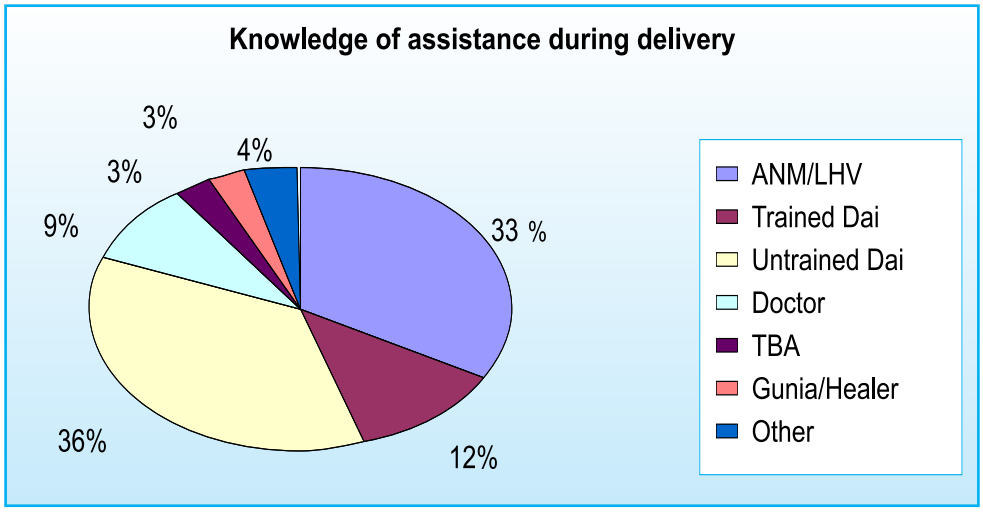

Figure 2: Knowledge of assistance during delivery

Knowledge of Postnatal care: Most of the female deaths occur during or immediately after birth.[16] Postnatal period is the period beginning immediately after birth of a child and extending for about 6 weeks or the return of the reproductive organs to their normal non-pregnant state. This period is very crucial to both mother and newborn baby and must be evaluated by doctor or ANM within the post natal period. All pregnant women need to be aware of the post natal care. However, only 40% Baiga women had knowledge of postnatal care services.

Knowledge on child Immunization: Immunization programs are among the most beneficial health interventions. Women who are considering pregnancy or who are pregnant need health care and obstetrical care. These health care providers are well placed to review the immunization status of the child and recommended vaccination strategies.[17] Government of India launched many programs for immunization/vaccination for better child health. It was seen that knowledge of immunization/vaccination is very poor among both rural and tribal inhabitants. Table 2 shows the awareness regarding BCG (51%), DPT (46%), OPV (54%), Vitamin-A (20%) and Measles (17%), etc among the target group. It was observed that most of the women did not knew the reason or use of immunization.

Table 2. Knowledge of child immunization

Knowledge of immunization	Yes (in %)
BCG	51
DPT	46
OPV	54
Vitamin-A supplement	20
Measles	17

Welfare programs for pregnant women: Government carries out many programs for the welfare of the pregnant women. In tribal areas, pregnant women are totally ignorant about these programs and as a result unable to gain the benefits of these programs. However, the currently married Baiga women had knowledge of government programs like Janani suraksha yojana (34%), ladali yojana (21%), national maternity benefit (5%), Deendayal card (57%), etc but failed to use them.

CONCLUSION

Poverty and ignorance seem to the main matter of concern among Baiga tribal women. The knowledge about maternal health care services among Baiga women is very poor as compared to the state as well as national averages. Despite, many incentives over 50% women still indicated lack of knowledge about institutional delivery, full ANC check up and assisted delivery. Therefore, there is an urgent need to spread the importance of antenatal check up, iron rich foods, institutional delivery, etc through IEC. Lastly, the indirect interventions like emergency health transport (Free pass to pregnant women), JSY and ASHA scheme will be effective in increasing the utilization of health services by these women.

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