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Original Aricle

An analysis of the attitude of Patients towards Laboratory Services for Malaria Disease in Hyderabad India

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ABSTRACT:

Malaria is the world's most important parasitic infection, which poses major health challenges. Malaria is a major cause of deaths in the tropical area of the world. Two hundred and nineteen million cases were reported worldwide in 2010. Changes in blood cell counts are a well-known feature of malarial infections. These changes involve major cell lines including red blood cells (RBC), leukocytes and thrombocytes. Human malaria can be caused by any of several species of Plasmodium parasites that occur together in various combinations in regions of endemicity. P. falciparum is responsible for almost all mortality attributed directly to malaria and is the focus of almost all research and intervention efforts. Compared with P. falciparum, however, P. vivax is the source of as much or more morbidity worldwide. Plasmodium spp. are parasites of blood and thus are expected to induce changes in blood however, some hematological changes associated with malaria are recognized while there are conflicting reports on others hematological parameters. This study aimed at the identification of plasmodium specie responsible for malaria and measuring changes in hematological parameters in malaria patients; to determine the causative specie of malaria to spot changes in hematological parameters in malaria patients; to study association between plasmodium specie and changes in hematological parameters in malaria patients. The study investigated the association between malarial parasite and changes in hematological parameters. This study has revealed the occurrence of malaria by specific plasmodium species and their prevalence. This study reveal the existence of any significant association between plasmodium species and hematological parameters in malaria patients. This study will provide basis for future researcher studying malaria. The findings of this study will help public health authorities in controlling malaria epidemics in area of this study.

Keywords: Patients' attitude, Laboratory Services, Malaria, Analysis Causative Species.

Introduction

Malaria is a disease caused by the bite of a female mosquito. Despite years of continual efforts, malaria is still a threat to over two billion people, representing approximately 40% of the world's population (WHO, 2012). Geographical distribution of the disease is worldwide, it is found in tropical areas, throughout Sub-Saharan Africa, and to a lesser extent in Southeast Asia, South Africa, the Pacific Islands, India and Central, and South America (Igbeneghu *et al.*, 2013). Best estimates currently describe the annual global burden of malaria as 300-500 million cases and 1-2 million deaths (WHO, 2005). It is an important protozoan infection responsible for 1.5 - 2.7 million deaths (Lal *et al.*, 2010). The laboratory services in adequately detecting based on which treatment of the sick patient starts is very important aspect in healthcare services. This study was conducted to identify the plasmodium specie which is responsible for malaria inter alia to measure the changes in hematological parameters in malaria patients. Further the study has been conducted to determine the causative specie of malaria to spot changes in hematological parameters in malaria patients; and what is the study relationship between plasmodium specie and changes in hematological parameters in malaria patients. The study investigated the association between malarial parasite and changes in hematological parameters. This study has found that the incidence of malaria caused by specific plasmodium species and their prevalence; it further reveals the existence of any significant association between plasmodium species and hematological parameters in malaria patients. This study will provide basis for future researcher studying malaria. The findings of this study will help public health authorities in controlling malaria epidemics in area of this study.

Literature Review

Causative Species

The early diagnosis of malaria is the key feature for its prompt treatment and prevention of complications which may include coma, hypoglycemia, acidosis, renal failure or pulmonary. Edema Microscopic diagnosis is needed for confirmation of malaria, but it requires technical expertise and at times may be unreliable when poorly executed (WHO, 2002). Six plasmodial species present a significant health threat for humans; Plasmodium falciparum is usually considered the most important in terms of deaths. P. vivax is a major cause of illness across large parts of the world, and it is increasingly argued that deaths, due to this parasite, have been underestimated (Naing *et al.*, 2014). P. ovale curtisi, P. ovale wallikeri, and P. malariae are fewer common causes of significant disease. Recently the simian parasite P. Knowlesi emerged as a local but important cause of disease (including severe disease) in Malaysia and other areas of southeast Asia, where it is predominantly a zoonosis with no definite evidence of primary human-to-human transmission (Ahmed & Cox-Singh, 2015). Historically P. falciparum has probably exerted greater selective pressure on human evolution than any other pathogen. Despite P. falciparum's presence throughout the tropics, the health impact is far from even, with the large majority of the world's parasitized individuals in Asia and south Asia (reflecting the significant human population) and 90% of deaths occurring in Africa, mostly in children (WHO, 2015).

Pathogenesis

Plasmodium spp. are global pathogens with a complex life cycle alternating between female Anopheles mosquitoes and vertebrate hosts that require the formation of unique zoite forms to invade different cell types at specific stages (Figure 1). Once sporozoites enter the host, they infect hepatocytes; this is followed by the asexual cycle in the blood. A feeding mosquito, completing the cycle, ingests sexual forms that develop during the blood stage (McKenzie *et al.*, 2008). This study concentrates specifically on the stages within the human host, in particular the liver and blood stages (Cowman *et al.*, 2016). Plasmodium sporozoites are injected into the host dermis during a blood feed. The fate of these sporozoites not well understood but they can take 1–3 hr. to exit from this site. Here, they rely on gliding motility, a random process enabling a proportion to reach and penetrate a blood vessel to enter the bloodstream. Those remaining in the skin can be destroyed and are drained by the lymphatic, where a host immune response is generated. The protein Trap-like protein (TLP) plays a role in exit from the dermis, as mutant sporozoites lacking its function display normal gliding motility but cannot enter the circulation. Those that enter the blood stream quickly access the

liver by a process known as traversal. This involves crossing the sinusoidal barrier comprising fenestrated endothelial cells and macrophage-like Kupffer cells (Regev-Rudzki *et al*., 2013). Cell-cell communication between malaria-infected red blood cells occurs via exosome-like vesicles. Cell proteins required for traversal include SPECT (sporozoite microneme protein essential for traversal (Ishino *et al*., 2004).

Immunity

Cell-passage activity is required for the malarial parasite to cross the liver sinusoidal cell layer. SPECT2 (also known as perforin-like protein 1, PLP1), CelTOS (cell traversal protein for ookinetes and sporozoites), phospholipase (PL) are proteins known to help in this activity (Bhanot *et al*., 2005). A surface phospholipase is involved in the migration of plasmodium sporozoites through cells and gamete egress and sporozoite traversal protein (GEST). The function of these proteins in cell traversal is not understood, although SPECT2 has a membrane attack complex/perforin-like (MAC/PF) domain, suggesting that it plays a role in punching holes in membranes. Sporozoites traverse through cells by forming a transient vacuole and SPECT2 and pH sensing is involved in egress from this structure (Risco-Castillo *et al*., 2015). Malaria sporozoites traverse host cells within transient vacuoles. It has been suggested that cell traversal through the sinusoidal barrier is important for infectivity by priming the sporozoite for invasion of hepatocytes, the cells in which sporozoites develop. However, the primary role of sporozoite traversal is crossing the sinusoidal barrier (Formaglio *et al.,* 2013). Sporozoites injected into the dermis are in ‘‘migratory mode’’ and upon interaction with hepatocytes convert to ‘‘invasive mode.’’ One signal for this switch is recognition of hepatocytes through binding higher sulfated forms of heparin sulfate proteoglycans (HSPGs) activating calcium-dependent protein kinase 6 (CDPK6) (Coppi *et al*., 2007). The tetraspanin CD81 and scavenger receptor B1 (SR-B1) are human hepatocyte surface proteins required for invasion and formation of a parasitophorous vacuole by P. falciparum sporozoites (C.D. Rodrigues et al., 2008). In contrast, the hepatocyte receptor EphA2 is not required for hepatocyte invasion but for intra-hepatocytic development by establishment of the parasitophorous vacuole through interaction with parasite proteins p52 and p36 (Mikolajczak *et al.*, 2015). A dense coat covers the sporozoite, and a key protein is the Circumsporozoite protein (CSP), consisting of a highly repetitive region and a type I thrombospondin repeat (TSR). Invasion of hepatocytes requires binding of CSP to highly sulfated proteoglycans (HSPGs), activating processing of CSP and removal of the N terminus exposing the TSR domain (R. Herrera et al., 2015).Subsequent steps involve proteins, including thrombospondin-related anonymous protein (TRAP) and apical membrane antigen-1 (AMA-1), with adhesive domains released from the apical organelles (micronemes and rhoptries). Once hepatocyte infection is established the sporozoite transforms over the subsequent 2–10 days to a liver stage (LS) or exo-erythrocytic form (EEF), and development culminates in release of up to 40,000 merozoites per hepatocyte into the bloodstream by budding of parasite-filled vesicles called merosomes (Sturm *et al*., 2006).Investigation of P. falciparum EEF transformation in vivo has been enabled by availability of human liver chimeric mice providing a crucial window into previously unknown biology DNA replication begins day 2 post-invasion, and parasites remain within a parasitophorous vacuole membrane into late LS development. (Vaughan *et al*., 2012).Once released into the hepatic circulation, free merozoites invade erythrocytes in a fast, dynamic, and multi-step process including pre-invasion, active invasion, and echinocytosis (Weiss *et al*., 2015). Pre-invasion is the initial interaction of merozoites with erythrocytes, and little is understood about the molecular details of this step. Merozoite surface protein 1 (MSP1) is the major glycophosphatidylinositol (GPI)-associated protein on the Merozoite surface (A.A. Holder, 1994). MSP1 acts as a platform on the Merozoite surface for at least three large complexes with different extrinsic proteins that bind erythrocytes (Lin *et al*., 2016). Merozoites lacking surface MSP1 can invade erythrocytes, suggesting that it is not required for invasion. It is possible that MSP1 is involved in display of proteins involved in evasion of host responses rather than directly in Merozoite invasion (Das *et al*., 2012).

Malaria and Hematological Parameters

Hematological changes in the course of a malaria infection, such as anemia, thrombocytopenia and leucopoenia are well recognized. These alterations vary with the level of malarial endemicity, background hemoglobinopathy, nutritional status, demographic factors, and malaria immunity. The World Health Organization (WHO) has listed

Hyperparasitemia as one of the criterion of severe falciparum malaria for more than two decades. Previous studies have shown that there is a correlation between parasite density and severity of malarial infections. Mortality is also correlated with the degree of parasitemia. Patients with the highest parasite densities also have the highest fatality rates. Additionally, high parasitemia due to Plasmodium falciparum infection takes a serious turn in anemia. Moreover, excessive hemolysis of parasitized RBCs in malaria infection may lead to anemia. Thrombocytopenia was also seen in the majority of patients with malaria. It was also observed that at high parasitemia, the platelets were found to be significantly lower. It has been noted by previous studies that increasing levels of P. falciparum parasite loads results in a decreased platelet count (Kotepui *et al*., 2014).

Method

The data used in this study was taken from two tertiary care hospitals, in Hyderabad India. The sample size consisted of 100 patients diagnosed with acute malaria receiving health facility at tertiary care units. Acute malaria was defined as history of fever in the past 72 hours with presence of parasitemia. Ethical approval for this study was taken from ethical committee of Fever Hospital Hyderabad India. A sample of 5ml venous blood was drawn from each patient, which was collected into tubes coated with ethylene diamine tetra-acetic acid (EDTA). Blood samples were at 2 - 8ºC stored on temperature until further laboratory investigation. The CP and MP test materials were used including Disposable Syringe 3 ml, Alcohol Swab, Tourniquet, EDTA Tube, Blood mixer, Glass Slide, Slide spreader, Hematology Analyzer (Sysmex XP 300), Binocular Microscope (CX 21 LED Olympus), Oil immersion, Methanol, Giemsa stain, Disposable Gloves, Sunny-Plast, Tissue Paper, Stopwatch, and Slide Dryer. Likewise, Laboratory investigation, Blood CP, and Hematology Analyzer Principle were employed. The Sysmex XP 300 analyzer is a quantitative, automated hematology analyzer for in-vitro diagnostic use in screening patient populations in clinical laboratories. The Sysmex XP 300 Analyzer provides the following: CBC, Leukocyte 5-Part Differential (Diff), Reticulocyte (Retic), and Nucleated Red Blood Cell (NRBC) on whole blood. In 1956, Wallace Coulter described the Coulter Principle as follows: A suspension of blood cells is passed through a small orifice simultaneously with an electric current. The individual blood cells passing through the orifice introduce an impedance change in the orifice determined by the size of the cell. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell passes through the aperture, it shortly increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For purposes of counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of recorded pulses indicates particle count, and the size of the electrical pulse is proportional to the cell volume. In addition, the system counts the individual cells and provides cell size distribution. The number of cells counted per sample is approximately 100 times greater than the usual microscope count, which reduces the odds of statistical error approximately 10 times.

Measurement of WBC

WBC/BASO Channel

RBCs are analyzed with the acid haemolytic reagent STROMATOLYSER-FB. This reagent selectively suppresses the degranulation of Basophils, resulting in their separation from other WBC. After this reaction the sample is analyzed by flow cytometry using a semiconductor laser to detect forward and side scattered light information, based on which a WBC/BASO scattergram is obtained. By analyzing this scattergram, WBC and Basophil counts were taken.

WBC 4 PART Differential

RBCs are analyzed with STROMATOLYSER-4DL. At the same time the reagent acts on the WBC membrane to allow dye passage. STROMATOLYSER-4DS (dying solution) is then added to allow the dye to enter WBC at the damages portion of its membrane and stain the DNA and RNA therein. Following this reaction, the sample is then analyzed by flowcytometry using the s semiconductor laser to detect forward and side scattered light information, based on which a 4-DIFF scattergram is obtained. By analyzing this scattergram, 4-parameter counts (lymphocytes,

monocytes, Eosinophils, Neutrophils and Basophils are taken. The immature information (IMI) channel of the XP-300 counts human progenitor cells (HPC). The reagents specifically affect the lipid components of the cell membranes; themembranes of mature cells, with a higher content of lipid are analyzed while immature cells retain their membranes. In normal samples, no intact cells are seen in the IMI area. The IMI channel is used to discriminate between immature and mature white blood cells. It utilizes the RF/DC detection method.

Measurement of RET and Optical measurement of PLT

Blood is diluted with RET SEARCH (II) to pre-set concentration. RET SEARCH (II) staining solution is also added to stain WBC DNA, RNA, and RET RNA. The sample is then analysed by flow cytometry using semiconductor laser to detect forward scattering light and side fluorescence information, based on which an RET scattergram is obtained. By analysing this scattergram, RET counts, RET ratios for individual fluorescence intensity zones (LFR, MFR, HFR), immature reticulocyte fraction (IRF) and PLT-O are determined. Platelets are also measured with sheath flow DC detection method. By measuring larger number of platelets, this method offers increased accuracy. However, in cases where many RBC fragments or large Platelets are present, accuracy tends to decrease. For such samples, the PLT-o measurement ensures higher accuracy than the DC detection method. XP-300 presents the platelet counts of high accuracy by adopting the results from either method that seems more accurate judging from the cell distributions and the scattergrams obtained.

Malarial Parasite (MP)

Blood samples were retrieved from blood storage system and subjected to screening for malarial parasite presence. Diagnosis of malaria involves identification of malaria parasite or its antigens/products in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors. The different forms of the four malaria species i.e., the different stages of erythrocyticschizogony; the endemicity of different species; the population movements; and the inter-relation between the levels of transmission, immunity, parasitemia, and the symptoms; the problems of recurrent malaria, drug resistance, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues; and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis can all have a bearing on the identification and interpretation of malaria parasitemia on a diagnostic test. The diagnosis of malaria is confirmed by blood tests and can be divided into microscopic and non-microscopic tests.

Microscopic Tests

The microscopic tests involve staining and direct visualization of the parasite under the microscope. For more than hundred years, the direct microscopic visualization of the parasite on the thick and/or thin blood smears has been the accepted method for the diagnosis of malaria in most settings, from the clinical laboratory to the field surveys. The careful examination of a well-prepared and well-stained blood film currently remains the “gold standard” for malaria diagnosis. The most commonly used microscopic tests include the peripheral smear study and the Quantitative Buffy Coat (QBC) test. The simplest and surest test is the time-honored peripheral smear study for malarial parasites. None of the other newer tests has surpassed the ‘gold standard’ peripheral smear study.

Peripheral Smear Study for Malarial Parasite (MP Test)

Light microscopy of thick and thin stained blood smears remains the standard method for diagnosing malaria. It involves collection of a blood smear, its staining with Romanowsky stains and examination of the Red Blood Cells for intracellular malarial parasites. Thick smears are 20–40 times more sensitive than thin smears for screening of Plasmodium parasites, with a detection limit of 10–50 trophozoites/µl. Thin smears allow one to identify malaria species (including the diagnosis of mixed infections), quantify parasitemia, and assess for the presence of schizonts, gametocytes, and malarial pigment in neutrophils and monocytes. The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia. The efficiency of the test depends on the quality

of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear. The test takes about 20 to 60 minutes depending on the proximity of the laboratory and other factors mentioned above. It is estimated to cost about 12 to 40 US cents per slide in the endemic countries. Before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000× should be examined on both thick and thin smears, which has a sensitivity of 90%. The level of parasitemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood. In non-falciparum malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (>50%) in falciparum malaria. In nonimmune individuals, hyperparasitemia (>5% parasitemia or >250 000 parasites/µl) is generally associated with severe disease. The smear can be prepared from blood collected by venipuncture, finger prick and ear lobe stab. In obstetric practice, cord blood and placental impression smears can be used. In fatal cases, post-mortem smears of cerebral grey matter obtained by needle necropsy through the foramen magnum, superior orbital fissure, and ethmoid sinus via the nose or through fontanelle in young children can be used.

Preparation of Smear

- Take a clean glass slide. Take 3 drops of blood 1 cm from the edge of the slide, take another drop of blood one cm from the first drop of blood.
- Take another clean slide with smooth edges, use it as a spreader, and make thick and thin smears. Allow it to dry.
- Slide number can be marked on the thin smear with a lead pencil.

Think Smear: The thick smear of correct thickness is the one through which newsprint is barely visible; it is dried for 30 minutes and not fixed with methanol. This allows the red blood cells to be hemolyzed and leukocytes and any malaria parasites present will be the only detectable elements. However, due to the hemolysis and slow drying, the plasmodia morphology can be distorted, making differentiation of species difficult. Thick smears are therefore used to detect infection, and to estimate parasite concentration.

Thin smear: Air-dry the thin smear for 10 minutes. After drying, the thin smear should be fixed in methanol. This can be done by either dipping the thin smear into methanol for 5 seconds or by dabbing the thin smear with a methanol-soaked cotton ball. While fixing the thin smear, all care should be taken to avoid exposure of the thick smear to methanol.

Staining: A number of Romanowsky stains like Field's, Giemsa's, Wright's, and Leishman's are suitable for staining the smears. Thick films are ideally stained by the rapid Field's technique or Giemsa's stain for screening of parasites. The sensitivity of a thick blood film is 5-10 parasites/µl. thin blood films stained by Giemsa's or Leishman's stain are useful for specification of parasites and for the stippling of infected red cells and have a sensitivity of 200 parasites/µl. The optimal pH of the stain is 7.2.Slides should be clean and dry. It is better to use neutral distilled water.

Thick Films: The thick film is first de-hemoglobinised in water and then stained with Giemsa.
Rapid Giemsa: Prepare a 10% Giemsa in buffered water at pH 7.1. Immerse the slide in the stain for 5 minutes. Rinse gently for 1 or 2 seconds in a jar of tap water. Drain, dry, and examine.
Standard Giemsa: Prepare a 4% Giemsa in buffered solution at pH 7.1. Immerse the slide (at least 12 hours old) in stain for 30 minutes. Rinse with fresh water, drain, dry, and examine.
Thin Films: Thin film examination is the gold standard in diagnosis of malarial infection.
Giemsa Stain: Fix with 1-2 drops of methanol. Cover the film with 10% Giemsa stain and leave for 30 minutes, wash with distilled water, drain, dry, and examine.

Figure 1

Differentiation of Malaria Parasites

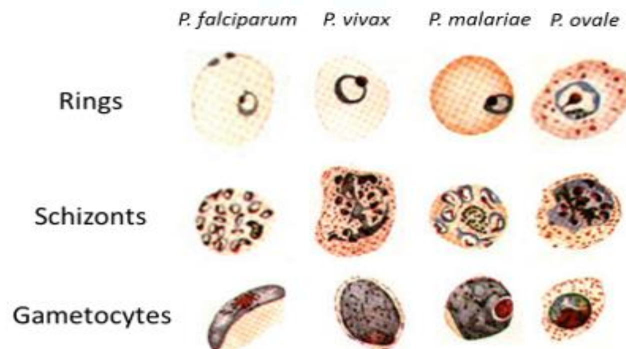


Table 1

Malarial Causes with Findings

Findings	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*
RBC Size	Not enlarged	Enlarged	Not enlarged	Enlarged
RBC Shape	Round, sometimes crenated	Round or oval, frequently bizarre	Round	Round or oval, often fimbriated
RBC Color	Normal, but may become darker; may have a purple rim	Normal to pale	Normal	Normal
Stipling	Maurer's spots appear as large red spots, loops, and clefts; up to 20 or fewer.	Schuffner's dots, appear as small red dots, numerous.	Ziemann's dots, few tiny dots, rarely detected	Schuffner's dots (James's dots). Numerous small red dots.
Pigment	Black or dark brown; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden-brown granules scattered through the cytoplasm	Black or brown coarse granules; scattered	Intermediate between *P. vivax* and *P. malariae*
Early trophozoite (ring)	Smallest, delicate; sometimes two	Relatively large; one chromatin	Compact; one chromatin dot; single	Compact; one chromatin dot; single

	chromatin dots; multiple rings commonly found	dot, sometimes two; often two rings in one cell		
Schizont	Medium size; compact; numerous chromatin masses; coarse pigments; rarely seen in peripheral blood	Large; amoeboid; numerous chromatin masses; fine pigments	Small; compact; few chromatin masses; coarse pigments	Medium size; compact; few chromatin masses; coarse pigments
Gametocyte	Crescent shaped, larger and slender; central chromatin	Spherical; compact	Similar to *P. vivax*, but smaller and less numerous	Like *P. vivax*, but smaller

Findings

Figure 2
Gender Distribution

Out of hundred samples (n=100), forty-four were female patients and fifty-six were male patients.

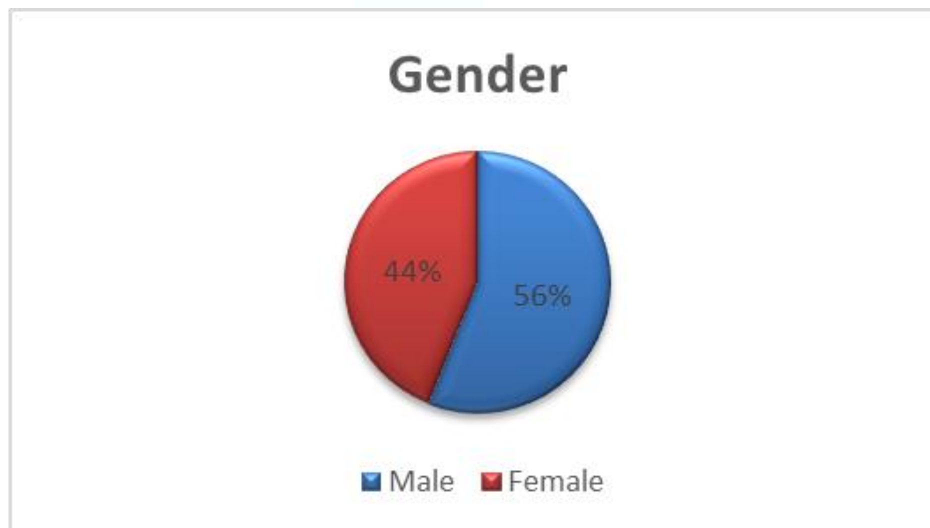
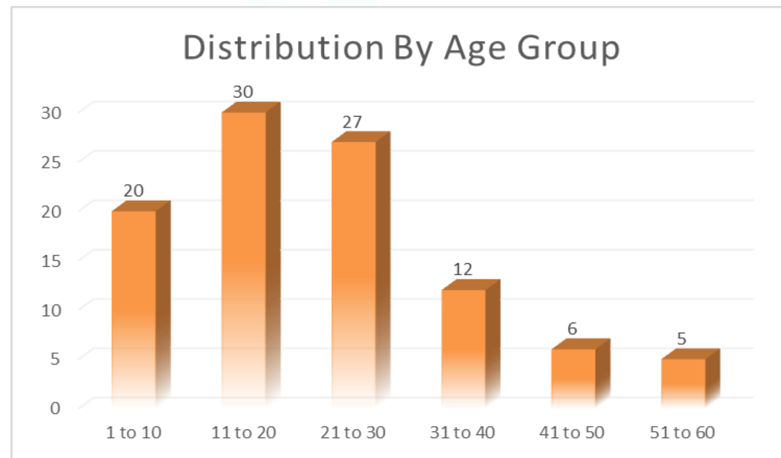


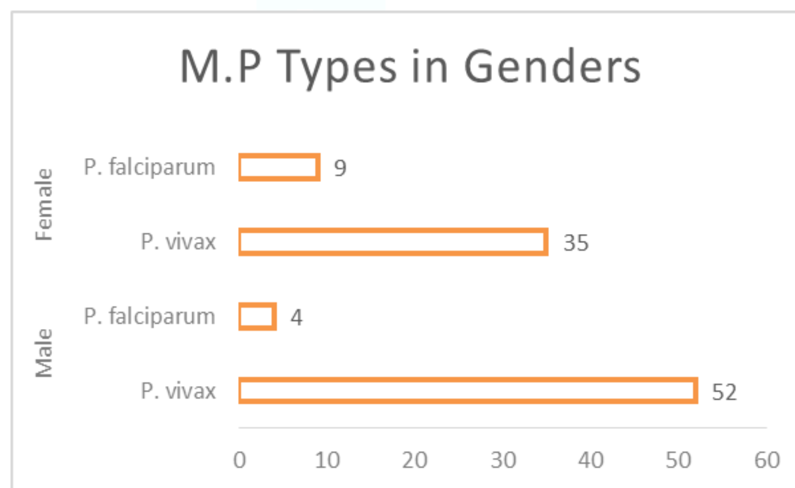
Figure 3
Age Group Distribution



Age group wise distribution of data revealed that highest number of malaria patients were between 11 to 20 years of age whereas lowest numbers of patients were above 40 years. Children between ages 1 to 10 contributed 20%, which is a considerable proportion.

Out of 100 malaria cases, 13 (nine females, 4 males) were caused by *Plasmodium falciparum* and 87 (35 females, 52 males) were caused *by Plasmodium vivax.*

Figure 4
Species Distribution in Gender Groups



Gametocyte and trophozoite distribution

Table 2

Among 87 cases of P. Vivax, 29 had gametocytes, one had trophozoites and 60 had both.

	P. Vivax		
	Gam	Tro	Gam/Tro
Male	18	0	34
Female	8	1	26

Table 3

Among 13 cases of P. falciparum, six had gametocytes, 3 had trophozoites and 1 had both.

	P. falciparum		
	Gam	Tro	Gam/Tro
Male	0	3	1
Female	6	0	3

Out of nine female cases of *P. falciparum*, one patient had leukopenia.

Figure 5

Plasmodium Falciparum effects on Gender Groups

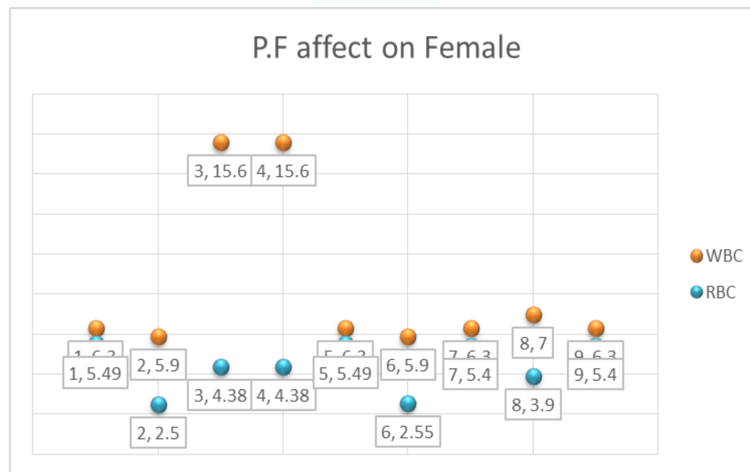


Figure 5

P. falciparum did not affect WBC count in females.

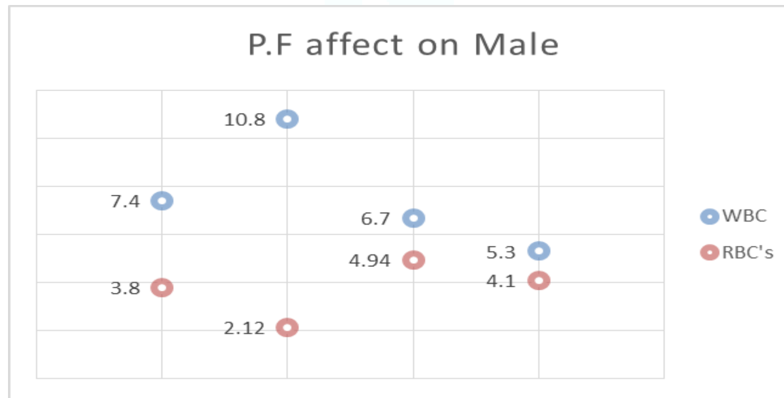
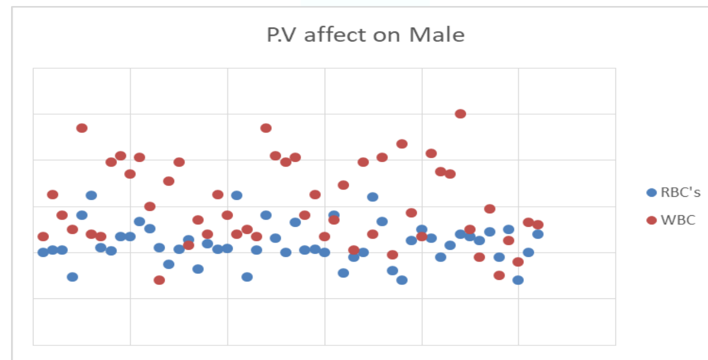


Figure 7

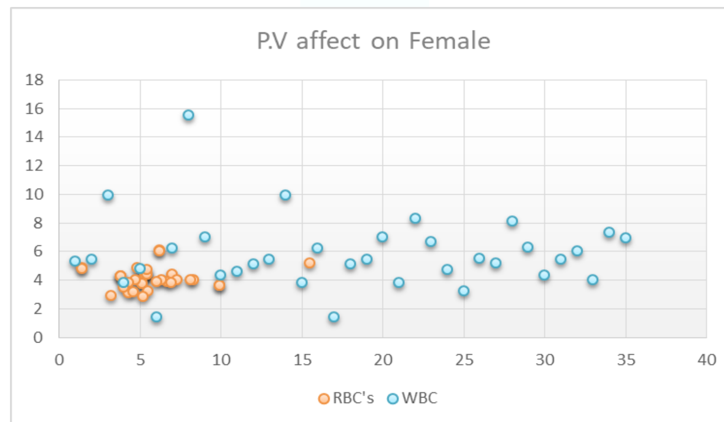
Plasmodium Vivax effects on Gender Groups



Out of 35 female cases of *P. vivax*, five had leukopenia.

Figure 8

PV effect on Female



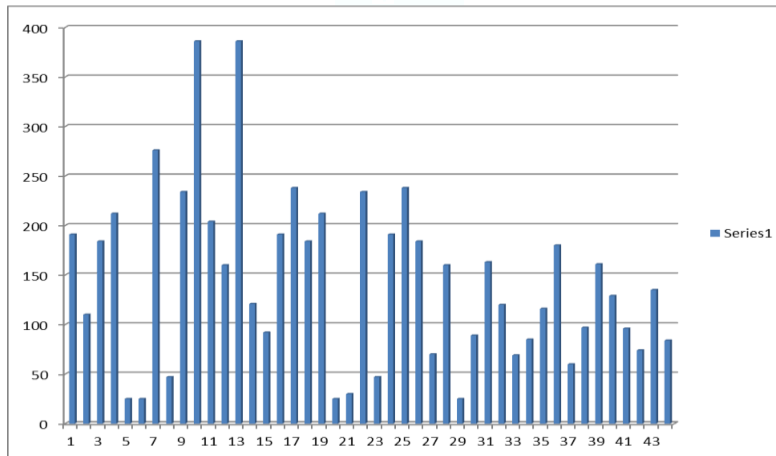
Out of 52 male cases of *P. vivax,* five had leukopenia.

Among 44 female patients of malaria, only four patients had normal HB level and rest of the 40 had anemia, 21 had normal platelets count and 23 had thrombocytopenia. Out of 56 male patients, 24 had normal HB level and 32 had anemia, 21 had normal platelets count and 35 had thrombocytopenia.

Platelet count in Gender Groups

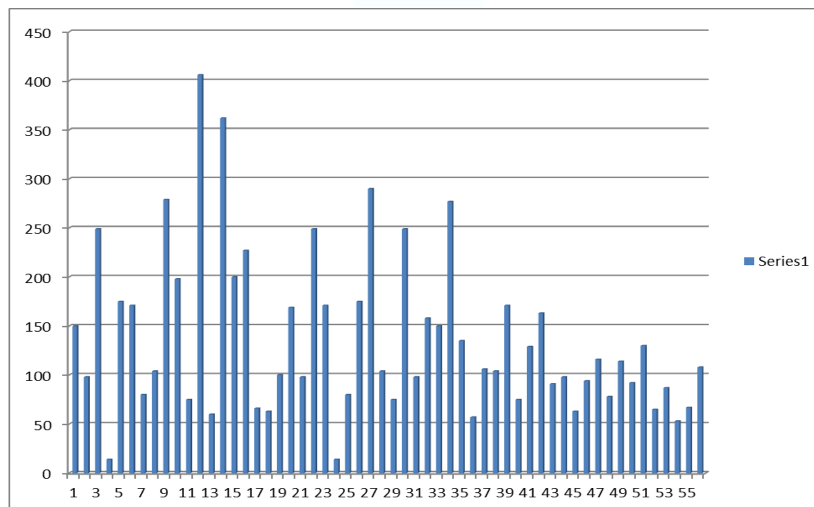
Out of 44 female patients, 21 had normal platelet count and 23 had thrombocytopenia.

Figure 9
Female patient's platelets count



Out of 56 male patients, 21 had normal platelets count and 35 had thrombocytopenia.

Figure 10
Male patients' platelets count



Discussion

Peripheral blood changes such as leukopenia, anemia and thrombocytopenia had been associated to malaria in previous study of Igbeneghu *et al.* (2018). The findings of this study showed that anemia and thrombocytopenia was associated with malaria in this study however, severe cases were not observed. On the other hand, leukopenia is not much evident among malaria patients. A previous study in Nigeria stated that the impact of malaria on hemoglobin level appears high in regions of sub-Saharan Africa because of underlying anemia and poor nutrition. In Eastern and Southeastern Asia where malaria also endemic, only small degree of hemoglobin concentration change is associated with malaria because underlying anemia and poor nutrition are not common. Anemia in acute malaria is due to increase in hemolysis and decrease in the rate of production of red blood cells, increased destruction of parasitized red blood cells and accelerated removal of both parasitized and unparasitized red blood cells. Other factors contributing to anemia in malaria include increased red blood cell deformability, spleenic phagocytosis and pooling (Igbeneghu *et al.,* 2018).The findings of our study also show similarity with the above stated study in finding anemia in large number of patients because of prevailing poor health and nutrition conditions in this area. The association between malarial parasite and anemia has remained inconclusive and conflicting reports exist on anemia in malaria patients. Previous studies stated that anemia could be considered as a measure of the cumulative impact of malaria on an individual patient. Patients with a prolonged history of fever (>2days) were 1.5-fold more likely to anemia and showed a 3-fold reduction in their hematocrit >25% (RN Prince *et al*., 2001). In another previous study, RBC counts, and hemoglobin were significantly reduced in high parasitemia patients. The mechanism-involving anemia is inconclusive but two possible causes of these alterations are increased hemolysis or a decreased rate of erythrocyte production (Kotepui *et al*., 2014). In this study, low platelet count was associated with malaria. Thrombocytopenia had been associated with malaria in previous studies and severe cases had been reported (Igbeneghu *et al*., 2018). The mechanism underlying thrombocytopenia associated with malaria is not well understood, however, it is believed to occur because of peripheral destruction and consumption. Immune complexes generated by malaria antigen lead to sequestration of the injured platelets by macrophages in the spleen, and platelet consumption in disseminated intravascular coagulation together with platelet dysfunction resulting in hyper-aggregation are thought to contribute to thrombocytopenia in malaria (Erhart *et al*., 2004). Moreover, a previous study showed that high parasitemia, even without complications, can lead to high mortality, which can reach to 50% in patients with parasitemia greater than 10% in areas of low transmission. This information is useful for public health agencies to specifically ease the malaria related anemia (Kotepui *et al*., 2014).

Conclusion

This study concludes that patients infected with malaria are prone to changes in hematological parameters with anemia and thrombocytopenia the two more important changes during malarial infection. In addition, the health condition and nutrition status of patients infected with malaria is also an extraneous variable in studying the impact of malarial infection on hematological parameters. The study investigated the association between malarial parasite and changes in hematological parameters. This study has revealed the occurrence of malaria by specific plasmodium species and their prevalence. This study reveals the existence of any significant association between plasmodium species and hematological parameters in malaria patients. This study will provide basis for future researcher studying malaria. The findings of this study will help public health authorities in controlling malaria epidemics in area of this study.

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