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Identification of *Plasmodium falciparum* in anemic patients with special reference to pregnancy

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Abstract

Anemia is a universal health hitch in developing as well as developed countries with main effects on human health as well as social and financial progress. Anemia is the decrease in the hemoglobin concentration of blood lower than normal range estimated for individual of different age and sex. Anemia is associated with parasitic infection caused by *Plasmodium falciparum*, a group of parasitic protozoan and transmitted by bite of female anopheles mosquito affecting 300 to 500 million new cases each year. In present study, a total of 50 anemic patients with suspected *Plasmodium falciparum* in their blood were studied from September to December 2015 in District Mardan, Khyber PukhtunKhwa, Pakistan. The microscopic examination showed overall positive result with 68% of anemic patients. It showed that female anemic patients having *Plasmodium falciparum* in blood were 46% and male anemic patients were 22%. The most vulnerable groups in this study were children with 42% and pregnant women with 16 % while the adult with 26% and non-pregnant women with 4% were recorded. It was concluded in the present study that *Plasmodium falciparum* was the causative agent of anemia in the project area.

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Introduction

Anemia is a condition that develops when your blood lacks enough healthy red blood cells or hemoglobin (Smith, 2009). Hemoglobin is a main part of red blood and binds oxygen (Rifkind & Nagababu, 2013). If a person have too few or abnormal red blood cells, or your hemoglobin is abnormal or low, the cells in your body will not get enough oxygen (Lefferts, 2014). Anemia is a universal health hitch in developing as well as developed countries with main effects on human health as well as social and financial progress (English *et al*., 1996). It is anticipated by World Health Organization (WHO) that the number of anemic people are about 2 billion and roughly 50% of all cases can be cause of iron deficiency (Stoltzfus & Dreyfuss, 1998). Anemia is accountable for about 1 million deaths per year, out of which three-quarters take place in South-East Asia and Africa (Stoltzfus & Dreyfuss, 1998).

Anemia is the decrease in the hemoglobin concentration of blood lower than normal range estimated for individual of different age and sex (Soyinka *et al*., 2008). The World Health Organization (WHO) defines that anemia is a condition in which hemoglobin value is below 13 g/dl in male more than 15 years of age, less than 12 g/dl in female over 15 years, and below 11 g/dl in conceived women (Mindell *et al*., 2013). It is a situation in which the concentration of red blood cells or their oxygen carrying capability is unsatisfactory to fulfill the physiologic requirements of body and this varies for gender, age and pregnancy status (Osungbade & Oladunjoye, 2012).

Anemia is associated with parasitic infection caused by *Plasmodium falciparum*, a group of parasitic protozoan and transmitted by bite of female anopheles mosquito (Perlmann & Troye-Blomberg, 1999) affecting 300 to 500 million new cases each year (Memon & Afsar, 2006). In Pakistan climate ranges changes from tropical to temperate, weather include high and low temperatures, rain fall and elevation of Pakistan ranges from zero to almost 9,000 meters (M. J. Bouma *et al*., 1996).

Among Plasmodium species *Plasmodium falciparum* are the widespread specie in Pakistan which causes about 36% of infections (Gething *et al*., 2012), and Plasmodium infections are found in the provinces of Baluchistan, Khyber PukhtunKhwa, Sindh and the Federally Administered Tribal Areas (Kakar *et al*., 2010). The main phase for transmission of *Plasmodium falciparum* is between August and December in Pakistan (M. Bouma *et al*., 1996). Anemia resulting from *Plasmodium falciparum* effect the people of all age and gender but the most effected groups are preschool children and pregnant women (Osungbade & Oladunjoye, 2012). Anemia constitutes a communal vigor problem in most of developing countries. Universally, about 2 billion inhabitants are expected to experience anemia and in South-East Asia and Africa it is estimated to account for three quarters of 1 million death per year (Osungbade & Oladunjoye, 2012). It is noticeable that the frequency of anemia in rising countries is about four times greater than industrialized countries (Allen & Gillespie, 2001).

In developing countries anemia affects over half of small children and conceived mothers, and in industrialized countries about 30-40% people become affected (Milman, 2011). Anemia in children are unfavorable as it affects their performance, behavior and mental growth. Children who experience anemia have low mental development and weak in learning; furthermore, they have impaired management of verbal communication and motor skills, comparable to a 5 to 10 points deficit in intelligent quotient (Osungbade & Oladunjoye, 2012). (Scanlon *et al*., 2000) performed an exposition legion investigation of HB level and birth result and found that anemia increased 70% risk of premature birth. *Plasmodium falciparum* was causative agent of anemia in children, along this an anemic mother gives birth to anemic baby which effect their cognitive performance, behavior and physical growth (Lone *et al*., 2004) and acquaintances have found that in anemic women threats of premature birth, low birth weight were more than non-anemic pregnant women. The female expecting anemia gave birth to babies who had 1.8 times increased possibility of having low APGAR (Appearance, Pulse, Grimace, Activity and Respiration) score at birth (Lone *et al*., 2004).

The most common and extensive communal health problem is anemia in pregnancy, affecting 24.8% of the population in world (Haniff *et al*., 2007). In developing countries 56% of all conceived mothers are anemic estimated by World Health Organization (WHO) (Organization, 1992). The prevalence of anemia in pregnancy is about 75% in Southern Asia as compared to North America and Europe with about 17% prevalence. Furthermore, 5% of pregnant women experienced severe anemia in highly prevalent countries of the world (Osungbade & Oladunjoye, 2012). Anemia has massive adverse consequences on women as the situation unfavorably affects both their productive and reproductive capabilities. First, anemia make them weak by reducing their energy and capacity for work (Awan *et al*., 2004). It is anticipated that anemia caused about 20% of maternal deaths; in addition, about 50% of all maternal deaths are caused by anemia (Galloway *et al*., 2002). Blood loss during or after childbirth leads to anemia which lowers the female hematological reserve and makes women more susceptible to death, severe anemia also make a person more susceptible to infection due to lowered immunity, hemoglobin (Hb) level of less than 4 g/dl is associated with high risk of cardiac failure and death particularly during delivery or soon after, if punctual intervention is not instituted (Galloway *et al*., 2002).

The lifecycle of *Plasmodium falciparum* is complex. An infected female Anopheles mosquito start life cycle of *Plasmodium falciparum* by biting a healthy human (Manguin *et al*., 2008). During feeding female Anopheles mosquito injects sporozoites from its salivary gland in the blood stream of human from where it reaches to liver cells (hepatocytes) (Manguin *et al*., 2008). In liver cells the sporozoites differentiate and multiply in number, resulting in thousands of merozoites that releases from the liver cells. Then the merozoites attack red blood cells (erythrocytes) and undergo rapid multiplication, producing 12-16 merozoites within a schizont. The merozoites which released from red blood cells go on to attack further erythrocytes (Cowman & Crabb, 2006). Not all of the merozoites invade additional red blood cells, some differentiate into sexual forms, male and female gametocytes.

Female Anopheles mosquito ingests these gametocytes during a blood meal. The male and female gametocyte undergoes a rapid nuclear division inside the mid gut of female Anopheles mosquito, which produces eight flagellated male microgametes that fertilize the female macrogamete (Manguin *et al*., 2008) and result in formation of ookinete traverses the mosquito gut wall, ookinete encyst itself on the exterior of the gut wall as an oocyst. The oocyst ruptures to release hundreds of sporozoites into the mosquito body cavity, from where they migrate to the mosquito salivary glands and is ready to infect other humans and continue its life cycle (Manguin *et al*., 2008).

Anemia due to *Plasmodium falciparum* may cause reduced mental capacity, impaired cognitive development, and fatigue and in intense form can lead to death, especially in conceived women (Getachew *et al*., 2012). Anemia results in morbidity and death in falciparum infection (Dondorp *et al*., 1999). In endemic countries parasitic diseases such as *Plasmodium falciparum* have long been predictable as a vital cause of anemia (McDevitt *et al*., 2004). Over 40% of world population lives in areas where Plasmodium infection is common, these areas include Southeast Asia, Bangladesh, Africa, areas of Middle East, India, Pakistan, Central and South America (Kondrachine & Trigg, 1997). On this continent, the greatest mortality is associated with severe anemia, especially in preschool age children, in malaria endemic areas (Guerra *et al*., 2008). Pakistan is endemic for Plasmodium infections and incidence rate of infection is one case per thousand population (Mujahid & Arif, 1998).

The level of anemia caused by the different mechanisms diverge according to the pregnancy status, age, gender, anti-malarial immune status and genetic makeup of infected persons, and the confined endemicity of Plasmodium infection. In general, hemolysis of red blood cells is of greater magnitude in non-immune children exposing acute anemia due to *Plasmodium falciparum*, whereas the

process of erythropoiesis is not seen in individuals exposed to repeated or regular falciparum infection, although, in any one individual, a number of mechanisms are liable to function (Menendez *et al*., 2000). The development of acidosis take place as a result of anemia, and may be the principal reason of acidosis where malarial anemia is widespread, although metabolic acidosis can take place in children irrespective of severe anemia (Marsh *et al*., 1995). Blood transfusion can be a deliverance mediation for individuals experiencing severe anemia and is comparatively uncomplicated to manage. On the other hand, the accessibility and safety of blood for transfusion have been mainly uneven in malarial endemic areas.

Therefore the present study conducted for detection or Identification of Plasmodium falciparum in anemic patients with especially in pregnancy stage.

Materials and methods

Area of Intervention

Samples were collected from anemic patients with suspected *Plasmodium falciparum* in their blood. The study was carried out from September 2015 to December 2015 in District Mardan, Khyber PukhtunKhwa, Pakistan. Mardan is the 19th largest city of Pakistan. It is the second most popular city in the province located in the south west of the province at altitude of 283 (Fig. 1).

Fig. 1. Map of district Mardan, KPK, Pakistan.

Collection of Blood Samples

Blood samples were collected from both male and female anemic patients through sterilized syringes. The blood was poured into a test tube containing anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA) to prevent clotting of blood.

The laboratory for processing by automatic machine, to count their Hb level. The remaining blood on the finger was wiped with dry cotton. Those blood samples having Hb level less than normal are then tested for *Plasmodium falciparum* (Fig. 2).

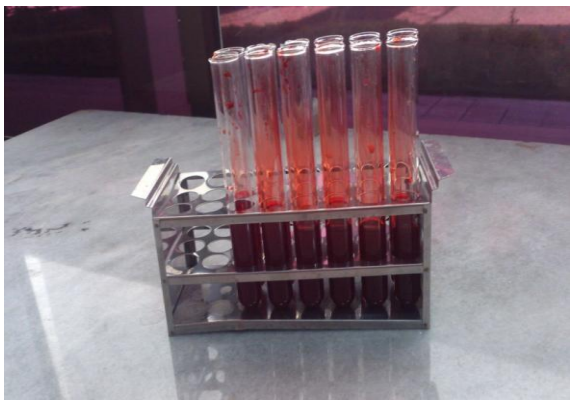

Fig. 2. Collection of Blood Samples in Test Tubes.

Blood Film Preparation

Thin and thick blood films were prepared for examination of *P. falciparum*.

The Thin Film

Thin film was prepared by falling a drop of blood on mid of slide. Place the slide in horizontal position with the blood on mid of plane and compact surface, and by means of another slide as spreader, touched the small drop of blood in mid of slide with the rim of the spreader, allowing the blood to spread along the border. Keeping spreader at an angle of 45 and pushing the spreader firmly along the slide (Fig. 3).

Fig. 3. Preparation of Thin Blood Smear.

The Thick Film

Thick film was prepared by falling few drops of blood on mid of slide with the help of edge of the spreader to link with the drops of blood on slide, and stretch them to make a smooth thick film. Do not whip the blood. The diameter of spherical thick film was about 1 cm (Fig. 4).

Fig. 4. Making of Thick Blood Smear.

Staining Blood Films with Giemsa Stain

Each thin film was fixed by sinking slide for a few seconds in a container having methanol. The slides were positioned back to back in a staining furrow, with no doubt that the thick films were on the whole at one end of the trough.

Giemsa stain solution was prepared of about 3% by mixing 3 ml of Giemsa stock solution with 97 ml of distilled water buffered to pH 7.2.

The stain was poured into the trough. Stained for 45-60 min.

Gently clean water was poured into the trough containing slides to float off the iridescent 'scum'. Gently poured off the remaining stain and rinsed with clean water.

The slides were cautiously removed from trough, one by one, inserting them in the drying rack to dry with film side facing downward.

Microscopic examination of blood film

For the presence and species identification of parasites microscopic examination of thin and thick blood films take place by using high power magnification using thin films under a x10 paired eyepiece and the objective of 100× oil immersion were carried out (Fig. 5).

Fig. 5. Microscopic Examination of Blood Smears.

Results

A total of 50 samples taken from anemic patients amongst those 31 were females and 19 were males. Out of 31 females, 16 were less than age of 5 and remaining 15 females were more than age of 5. In these 15 females, 10 females were pregnant and 5 were non-pregnant. Out of 19 male anemic patients 12 males were less than age of 5 and remaining 7 males were more than age of 5.

Overall Result

Both male and female anemic patients in samples showed positive results with 68% (34/50) while negative results were 32% (16/50).

Gender Wise Result

Female anemic patients showed positive result with 46% (23/31) while negative results were 16% (8/31). Male anemic patients showed positive result with 22% (11/19) while negative results were 16% (8/19).

Table 1. Gender-Wise Prevalence of *Plasmodium falciparum* In Anemic Patients in District Mardan, Khyber PukhtunKhwa.

Sex	Positive Numbers	%age
Male	11	22%
Female	23	46%
Total	34	68%

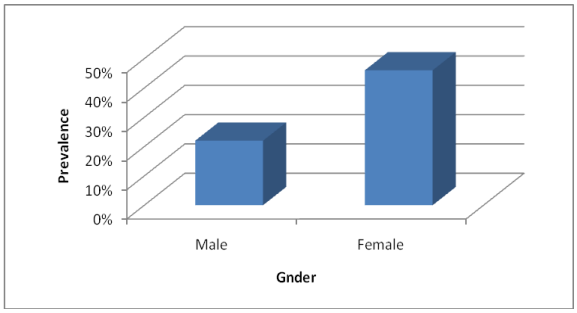

Fig. 5. Sex-Wise Prevalence of *Plasmodium falciparum* In Anemic Patients in District Mardan, Khyber PukhtunKhwa.

Age Wise Result of All Patients

Patients less than age of 5 showed positive result with 42% (21/28) while negative results were 14% (7/28). Patients more than age of 5 showed positive result with 26% (13/22) while negative results were18% (9/22).

Age Wise Result of Male Anemic Patients

Among male anemic patients with 22% positive result, patients less than age of 5 showed positive results with 16% (8/12), while showed negative result were 8% (4/12).Male anemic patients more than age of 5 showed positive results with 6% (3/7) while negative result were 8% (4/7).

Table 2. Age-Wise Prevalence of *Plasmodium falciparum* in both Male and Female Anemic Patients in District Mardan, Khyber PukhtunKhwa.

Sex	Positive %age		
	Age Below 5	Age Above 5	Total %age
Male	16%	6%	22%
Female	26%	20%	46%
Total	42%	26%	68%

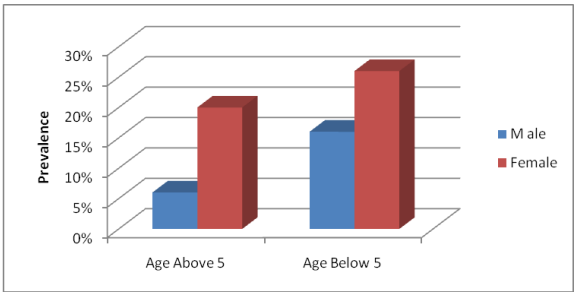

Fig. 6. Age-Wise Prevalence of *Plasmodium falciparum* in Both Male and Female Anemic Patients in District Mardan, Khyber PukhtunKhwa.

Pregnancy Wise Result

Pregnant female anemic patients showed positive result with 16% (8/10) while negative results were 4% (2/10). Non-pregnant female anemic patients showed positive result with 4% (2/5) while negative results were 6% (3/5).

Table 3. Pregnancy-Wise Prevalence of *Plasmodium falciparum* in Female Anemic Patients in District Mardan, Khyber PukhtunKhwa.

Pregnancy Status	Positive No	Positive %age
Pregnant	8	16%
NonPregnant	2	4%
Total	10	20%

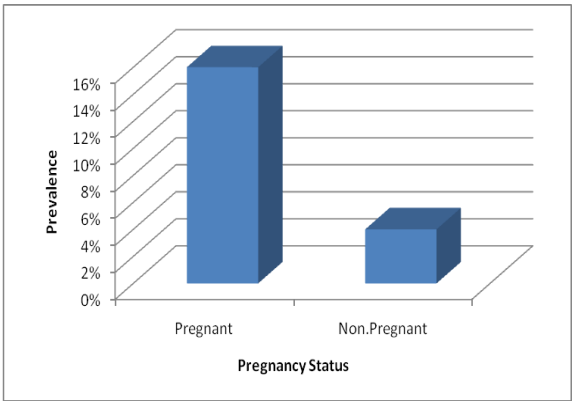

Fig.7. Pregnancy-Wise Prevalence Of *Plasmodium falciparum* In Female Anemic Patients Having Age More Than Five In District Mardan, Khyber PukhtunKhwa.

Discussion

Results of present study showed that *Plasmodium falciparum* is one of the causative agent of anemia as identified through microscopic examination. Anemia is prevalent in Mardan of KPK and has been reported from other provinces (2), where the causative agent of anemia is *Plasmodium falciparum*, a protozoan parasite. The present study report and confirm that anemia effecting people of all ages and gender in the study area, similar outbreak have been reported by (Baig-Ansari, 2008) in an urban area of Pakistan. The patients exposing anemia in childhood and late in pregnancy is common to see in Pakistan. The same is obvious from this study, where a large number of patients experiences anemic during pregnancy.

Baig-Ansari, 2008 found that in an urban setting in Pakistan about 90.5% conceived women were exposing anemia.(Stevens *et al*, 2013) found that in Pakistan in 1995 48% female were anemic during pregnancy, 47% were exposing anemia during 1996-2000, 46% during 2001-2002, 47% during 2003-2006, 48% during 2007-2008, 49% in 2009, 50% in 2010 and 51% during 2011-2013.

In this study blood taken from 50 anemic patients were examined for presence of *Plasmodium falciparum* by making thin and thick film and staining. The children were major susceptible groups in this study 42% (21/28), and pregnant women 16% (8/10), although other female and aged people are next affected. In Pakistan a study on prevalence of anemia, the report indicates that 96% of pregnant women were anemic in Multan area of Pakistan (2).

This study has established an informal association between anemia and maternal complications which leads to premature infant, low birth weight and babies with physical and mental retardation. The underlying cause is postulated due to presence of *Plasmodium falciparum* which causes anemia in pregnant women as well as new born child.

This study has emphasized the consequence of considering anemia because of having *Plasmodium falciparum* in blood especially in children and pregnant women expecting anemia is a sign of unfavorable outcome of birth. Consequently, to diminish the load of this difficulty, actions must be execute at community level, which can avoid and treat anemia in people of all age, sex and especially pregnant women and children. Anemia and Plasmodium infections awareness programs should be initiated by Health education authorities in schools and should be further promoted at the local level reaching out to all people. We conclude that this ignored distribution of anemia should be measured by *Plasmodium falciparum* control policy makers in the world and by the Pakistani government.

Conclusion

Anemia resulted from infection of *Plasmodium falciparum* is presently a severe health trouble of children and conceived women living in Mardan, Khyber PukhtunKhwa, Pakistan. It was concluded that *Plasmodium falciparum* was the causative agent of anemia in the study area. Anemia and Plasmodium infections awareness programs should be initiated by Health education authorities in schools and should be further promoted at the local level reaching out to all people. We conclude that this ignored distribution of anemia should be measured by *Plasmodium falciparum* control policy makers in the world and by the Pakistani government.

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