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RESEARCH ARTICLE

PREVALENCE OF MATERNAL ANEMIA DUE TO PLASMODIUM FALCIPARUM IN DISTRICT MARDAN, KHYBER PAKHTOON KHWA, PAKISTAN

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ARTICLE INFO	ABSTRACT
<i>Article History:</i> Received 09 th August, 2016 Received in revised form 28 th September, 2016 Accepted 20 th October, 2016 Published online 30 th November, 2016	Over 40% of the world population lives in area where plasmodium infection is common including Pakistan. Pakistan is endemic for Plasmodium infections and incidence rate is one case per thousand populations. These parasites not only decrease the Hemoglobin level but also reduce immunity. The present study was based on identification of anemia due to plasmodium among conceived women of district Mardan. In the present work a total number of 100 female conceived women blood was studied from April 2016 to June 2016 the microscopic examination showed that all most 100% of the
Key words:	female during pregnancy were suffering from anemia during one or other stage of their trimester however 64% showed positive result for <i>Plasmodium falciparum</i> at early or later phase of their
Plasmodium, Infection, Conceived, Trimester, Gestation period.	trimester and only 34% women were anemic only during their gestation period. It was recorded from the recent study that <i>Plasmodium falciparum</i> was one of the major cause of anemia in pregnancy in the selected area. The positive record indicates that plasmodium infection during pregnancy increase risk of low birth weight of fetus and can increase death rate during the first year of life.

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INTRODUCTION

The most common and extensive public health problem is anemia in pregnancy, affecting 24.8% of the population in world (Haniff et al., 2007; Yajnik et al., 2010). In developing countries 56% of all conceived mothers are anemic estimated by World Health Organization (WHO) (De Maeyer and Adiels, 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 and 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 (Axemo et al., 1995). 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

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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 (Ross et al., 1996; Osungbade and Oladunjoye, 2012). 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 and Trigg, 1997). On this continent, the greatest mortality is associated with severe anemia, especially in preschool age children, in malaria endemic areas (Gikandi et al., 2008; Guerra et al., 2010). Pakistan is endemic for Plasmodium infections and incidence rate of infection is one case per thousand population (Mujahid and Arif, 1998). The World Health Organization (WHO) reported that in Pakistan the percentage of Plasmodium infections credited to Plasmodium falciparum rose from 34% to 54% from 1987 to 1990 (Bouma et al., 1996; Parvez et al., 1996). The incidence of Plasmodium falciparum among

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microscopy-positive cases rose from 45% to 68% from 1995 to 2006 in the Quetta city of Baluchistan and in city of Jhangara in Sindh (Durrani et al., 1997; Rab et al., 2001). Plasmodium falciparum cause malaria which contributes to anemia which effect people of all age and especially during pregnancy. It is estimated that 23 million expecting women expecting plasmodium infection per annum in sub Saharan Africa and about 400,000 pregnant women exposed to moderate or severe anemia each year (hemoglobin < 80 g/L or hematocrit < 0.25) as a result of plasmodium infection in sub-Saharan Africa (Guyatt and Snow, 2001; Getachew et al., 2012). Negative consequences of Malaria in pregnancy (MIP) is an increased risk of low birth weight (LBW) for the fetus, which is usually reported (Wirima et al., 1996; Skette et al., 2001; Guyatt et al., 2004; De Beaudrap et al., 2013) which is further a considerable hazard developmental reason for disability (Teplin et al., 1991; Taylor et al., 2000; Grantham-McGregor S et al., 2007) and increases death rate during the first year of life (McCormick et al., 1985; Bardaji et al., 2011).

Numerous investigations found that low birth weight threat was linked exclusively with infections caused by Plasmodium falciparum during early stages of pregnancy (Kalilani et al., 2010; Huynh et al., 2011; Valea et al., 2012). In Asia anemia is responsible for 12.8% of maternal mortality which is second foremost reason of maternal mortality independent of death take place from postpartum hemorrhage (Khan et al., 2006; Sanghvi et al., 2010). In Bangladesh the nationwide survey estimated that prevalence of anemia was 50% (Yip et al., 2002; Noronha et al., 2012). In Pakistan (2001) the prevalence of anemia among conceived women ranged from 39.1% estimated from health nutrition and population statistics (Steer, 2000; Yip and Ramakrishnan, 2002). The adverse effects of anemia in pregnancy are increased infant mortality resulted from low birth weight as well as maternal mortality and morbidity (Steer, 2000; Noronha et al., 2012). The anemia of Plasmodium infection is complex, linking both the devastation of RBC and the production of low number of RBC's. 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, haemolysis 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; English et al., 1996). Blood transfution 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 (Fleming, 1997). The appropriate use of blood transfusion has been shown to trim down considerably mortality among conceived women experiencing severe anemia primarily caused by Plasmodium falciparum infectionsand folate deficiency (Brabin and Johnson, 2005). In the management of severe anemia, such as during pregnancy or in crude subjects with sickle-cell disease, a therapeutic anti-malarial that is if possible

not an antifolate should be recommended, followed by folic acid at 5 mg per day for three weeks (Stoltzuf and Dreyfuss, 1998).

The main objective of this study was: To identify Plasmodium *falciparum* in conceived mothers/pregnant woman which leads to anemia and other health issues in Mardan district Khyber Pakhtoon Khwa, Pakistan.

MATERIALS AND METHODS

Region of Intervention

The study was carried out from April 2016 to June 2016 at District Mardan, Khyber PukhtunKhwa, Pakistan. Mardan which 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 meters (928 ft.).

Blood Sample Collection

Blood samples were collected from conceived mothers. The anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA) was added to the samples to prevent clotting of blood. The samples were transported to the laboratory for processing by automatic machine, to count their Hemoglobin (Hb) level and for the presence of *Plasmodium falciparum*.



Figure 1. Collection of Blood Samplesin Test Tubes

Blood Film Preparation

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

The Thin Film

The aim of this film was to get monolayer. 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.

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.



Figure 2. Making Of Thin Blood Smear



Figure 3. Making Of Thick Blood Smear

Staining Blood Films with Giemsa Stain

Giemsa stain is a classic blood film stain for diagnosis of Plasmodium. 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 was carried out using high power magnification using thin films under a x10 paired eyepiece and the objective of $100 \times$ oil immersion werecarried out.(Warhurst dc, Williams 1996).

RESULTS

Overall Result

A total of 100 samples taken from subjects among those 36 subjects were suffering from anemia and 64 subjects showed positive result for *plasmodium falciparum* with addition to anemia.

Hemoglobin level Result

Pregnant female anemic patients showed positive result with 36% (36/100). The normal Hemoglobin level of adult female is 12 to 15.5 grams per deciliter of blood. In pregnant women a low hemoglobin level count is generally defined as less than 13.5 grams per deciliter of blood.

Plasmodium falciparum Result

Pregnant female anemic patients showed positive result with 64 (64/100) along with the presences of parasites in their blood (i.e. *plasmodium falciparum*).

Table 1. Prevalence of Plasmodium falciparum in Female Anemic Patients in District Mardan, Khyber Pakhtoon Khwa

Status	Positive Subjects	Positive Subjects %age
Pregnant(Anemic only)	36	36%
Pregnant(<i>P.falciparum</i>)	64	64%
Total	100	100%

DISCUSSION

Results of present study showed that Plasmodium falciparum a protozoan parasite is one of the causative agent of anemia in pregnancy as identified through microscopic examination. Anemia is prevalent in Mardan of KPK and has been reported from other provinces. Our study is parallel (Baig-Ansari, 2008) in an urban area of Pakistan. The present study/ report confirm that a large number of subjects experience anemia 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 100 female anemic patients were examined for presence of *Plasmodium falciparum* by making thin and thick film and staining. The pregnant women were major at risk groups in this study 64% (64/100), and 36% (36/100) are next affected, they were only anemic in pregnancy. In Pakistan a study on prevalence of anemia, the report indicates that 96% of pregnant women were anemic in Mardan area of Pakistan. This study has recognized as familiar connection between anemia and maternal complications which leads to early/premature child birth, low birth weight and brood 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 outcome of bearing in mind that anemia because of having *Plasmodium falciparum* in blood especially in pregnant women expecting anemia is a sign of unfavorable outcome of birth. Thus, to reduce the effect of this complexity, measures must be carrying out at community level, which can avoid and treat anemia.

Conclusion

Anemia resulted from infection of *Plasmodium falciparum* is currently a strict health danger of considered females living in Mardan, Khyber PukhtunKhwa, Pakistan. It was concluded that *Plasmodium falciparum* was the contributory cause of anemia in the study region. Anemia and Plasmodium infections awareness programs should be initiated by Health organizations and should be further promoted at the local level reaching out to all people .We finalized that this overlooked division of anemia caused by *Plasmodium falciparum* should be calculated by Health institute in the world and next to the Pakistani administration

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