Prevalence of Drug Resistance Malaria in Pakistan
(Plasmodium.vivax and P. falciparum)

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Abstract

Malaria every year kills millions of people in tropical areas across the world. The major obstacle to roll back malaria is drug resistance, thus conducting research is very important in this field. It’s known that drug efficacy determines by plasma separation and quantification and several factors play important role in it such as sensitivity, partial immune response and toxic effects. The poor cure rates due to substandard and fake anti-malarial drugs are another obstacle to roll back malaria in developing countries; substandard tablets contain poor action ingredients. The general aim of the present study is to assess the prevalence of drug resistance malaria (Plasmodium vivax and P. falciparum) in malaria endemic areas of Pakistan. This is a literature review study and it included published research papers on drug resistance malaria in Pakistan. Inclusion and exclusion criteria was used, Pubmed and Scopus data basis were used, eight papers were selected, coded, analyzed and critically discussed. Therapeutic efficacy tests involving chloroquine and other artemisin-combined therapy (ACTs) based on post-1973 WHO protocols found several factors which take part in emergence of decreased anti-malarial sensitivity. One factor is substandard tablets which contain poor action ingredients. In addition sub-therapeutic dosing increases drug resistance for malaria. In some regions other factors could be duration, artemisinins used from last few decades, unique massive drug pressure, low malaria transmission are facilitating anti-malarial sensitivity and resistance. Late diagnosis, lifestyle, male gender, traveling, poor diagnosis, vector control, education of medical practitioners and miss use of medicine are other significant factors for the prevalence of drug resistance malaria in Pakistan. Most of studies observed that children are more vulnerable to anti-malarial resistance (6-15 years age group), in addition male gender were more affected, which could be due to nature of their work. Progressive Artemisinin resistance P.falciparam malaria has been reported during routine surveillance. Despite progress in health system malaria still remains a major burden to public health. Its fact that incidence of P. falciparum is increasing compared with P.vivax. The major obstacle to roll back malaria in Pakistan is anti-malarial resistance and poor cure rates of anti-malarial drugs. The prevalence trend of P.falciparam has shown continuous increase in all endemic areas of Pakistan. Drug efficacy trials conducted in Pakistan has given important indication to guide country’s national malaria treatment policy. There is need of more extensive studies to observe, find obstacles and conduct drug resistance surveillance regarding anti-malarial in Pakistan. Further, there is need to monitor response of anti-malarial and take measures to prevent surfacing of drug resistance. Prevention of resistance could be possible through access of high quality effective ACTs in large scale. Furthermore there is need of proper education of practitioners, raise observance of ACTs users, proper diagnosis to avoid medicine misuse, transmission control to decrease use of anti-malarial drugs and diminish reservoir of infection. In Pakistani context encouragement of early diagnosis, proper treatment, reduce drug pressure, optimize vector control, target mobile population, proper management, good surveillance and operational research is important.

Keywords: Plasmodium, Vivax, Falciparm, Malaria, Drug resistance, Sulphadoxine pyrimethamine,

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INTRODUCTION
The earliest malaria parasite evidence was traced about one hundred million years ago (Paleocene period) in mosquitoes which have preserved in amber. The *Plasmodium falciparum* malaria was common among ancient Egyptians, prevalence was estimated 42%. Discovery disclosed after research on pre dynastic mummies in Egypt. There was no treatment for malaria in old ages, thus patients were badly suffered in ancient time (Thanh, 2012). Clinical symptoms of malaria were firstly described by Hippocrates about four hundred years ago (Gilles et al., 1991). Discovery of “Peruvian bark” was believed a major success for cure of malaria in seventeenth century. Major component of Peruvian bark was quinine and acted as schizontocidal (WHO “Bruce–Chwatt”, 1993). An Algerian army surgeon during late nineteenth century discovered the effects of malaria infection, the malaria parasite was found in red blood cells of human, later he received Nobel Prize on basis of this discovery (Gilles et al., 1991). World Health Organization (WHO) started a campaign in 1955 to eradicate malaria globally but still 1.6 million people across the world exposed to malaria infection and manifestations are in form of social and economic issues in developing world (Foster and Phillips, 1998). Malaria kills approximately 1.6 to 2.0 million people every year in the world (WHO, 1996). The economic effects due to malaria infection are worse and huge burden on societies. Approximately eight billion USD annually spent on malaria treatment in Africa and these huge expenses effects growth of economy and community activates in Africa (Foster and Phillips, 1998). Poor communities in tropical regions of the world are primarily affected by malaria infection and malaria infection became a major burden on public health system. Epidemiology and control interventions of malaria are not similar across the world, thus in malaria endemic countries the malaria infection slowdowns economic growth and development practice (WMA, 2000.; WHO, 2001). *Plasmodium* species can cause malaria infection in human and transmission vector is a female mosquito “Anopheles”, it injects parasite into human red blood cells (RBCs) and later release pyrogenic substances which can destroy RBCs. Malaria parasite has four *Plasmodium* species and most fatal one is *Plasmodium falciparum* (Gilles et al., 1991).

There is need to roll back malaria and ease burden on public health system but drug resistance is a major barrier to eradicate malaria. Chloroquine once considered most effective anti malarial drug and saved millions of human lives since 1950s but it’s not effective anymore for treatment of *P.falciparam* in tropical areas. The resistance against new anti malaria drug sulfadoxine pyrimethamine is much faster; it was introduced to replace chloroquine as anti malarial drug (WHO, 2004). Another anti malaria drug “Mefloquine” was introduced to combat with malaria infection but resistance observed within six years of introduction (Nosten, and Kuile, 1991). It’s a fact that malaria is a curable disease and on time accurate diagnosis can reduce the fatal effects of malaria infection. Malaria should be diagnosed using rapidly accurate method to reduce the human sufferings (Phillips et al., 2001).

MATERIALS AND METHODS
Study Design
This is a literature review study and it included published research papers on drug resistance malaria in Pakistan. Following scientific data basis were used in this scientific research study: Scopus, Pub Med, Science direct, Google scholar. The articles which fully fulfilled the including and excluding criteria of the study were selected. The well reputable data base “PubMed” and “Scopus” were used to search published articles on topic. Firstly the MeSh data base was adopted on Pub Med and then Scopus data base was used to search the synonyms; those are closely related to the exposure and outcome of the study. The both scientific data basis were approached through University of Oulu student account to get access to maximum unpaid scientific published articles on topic in Pakistan. Following key words/synonyms were used in search data basis: *Plasmodium*, vivax, falciparm, malaria, drug resistance, Sulphadoxine...
pyrimethamine, Chloroquine, antimalarial, malaria parasite, climate change, environmental health, vulnerable populations, weather, mortality, dengue, infectious diseases, refugees, Pakistan.

Identification of the study was carried out according to the inclusion and exclusion criteria and publications were included basis on fulfilled criteria. The wide including criteria were used to avoid missing any relevant publication and exclusion criteria were used during search data base and afterward to make sure to expel irrelevant publications.

RESULTS AND DISCUSSION

Burden of Malaria on Pakistan

Malaria is a mosquito borne parasitic disease, predominantly a disease of poverty because it’s more prevalent in slum areas where poverty rate is high and people have low economic status. The prevalence and incidence of malaria infection varies in each administrative unit of Pakistan. A research survey conducted in 19 endemic districts of Pakistan about malaria infection (Figure 1). Highest prevalence found in FATA region of Pakistan and followed by Baluchistan and then KPK (Rowland et al., 1999).

Table 1: Incidence of malaria parasite in Pakistan during 2004-09 (Rowland et al., 1999)

<table>
<thead>
<tr>
<th>Area</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>FATA</td>
<td>4.0(10)</td>
<td>4.5(19)</td>
<td>5.0(14)</td>
<td>6.2(9)</td>
<td>6.6(16)</td>
<td>4.0(10)</td>
</tr>
<tr>
<td>Baluchistan</td>
<td>4.2(14)</td>
<td>7.4(41)</td>
<td>6.2(19)</td>
<td>6.4(19)</td>
<td>5.0(16)</td>
<td>5.0(16)</td>
</tr>
<tr>
<td>KPK</td>
<td>1.1(8)</td>
<td>0.9(15)</td>
<td>0.8(9)</td>
<td>0.8(10)</td>
<td>0.8(11)</td>
<td>1.0(11)</td>
</tr>
<tr>
<td>Sindh</td>
<td>1.0(8)</td>
<td>0.6(3)</td>
<td>0.9(14)</td>
<td>0.7(12)</td>
<td>0.6(12)</td>
<td>0.6(12)</td>
</tr>
<tr>
<td>Punjab</td>
<td>0.0(1)</td>
<td>0.6(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
</tr>
<tr>
<td>Azad Jammu &amp; Kashmir</td>
<td>0.1(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
<td>0.0(1)</td>
</tr>
<tr>
<td>Total</td>
<td>17.0(19)</td>
<td>23.0(22)</td>
<td>20.0(10)</td>
<td>20.0(10)</td>
<td>18.0(19)</td>
<td>15.0(15)</td>
</tr>
</tbody>
</table>

Malaria Drug Resistance in Pakistan

In year 2001 Malaria Control Program, Ministry of Health Pakistan and World Health Organization planned to roll back malaria and bring malaria annual cases to 0.5/1000 till 2010 but proposed target became difficult to achieve (Rana et al., 2001). The major obstacle to roll back malaria in Pakistan is antimalarial resistance and poor cure rates of antimalarial drugs. First case of drug resistance malaria was reported during 1981 in
Pakistan, it was about chloroquine resistance in Shekapura district of Pakistani Punjab. In addition sulfadoxine pyrimethamine resistance is growing in Pakistan but artemisinin based combination therapy is considered more effective and it’s adopted as first line treatment for *P. falciparum* treatment (Khan et al., 1993; Rana et al., 2001).

**Therapeutic Efficacy Tests Involving chloroquine (Based On Post-1973 WHO Protocols)**

Since 1980s invivo studies were conducted based on WHO protocols for therapeutic efficacy testing in Pakistan to check anti-malarial efficacy and drug resistance. In Pakistan, first *P. falciparum* resistance to chloroquine was found in 1981 (Fox et al., 1985) and first study was published in 1985 which was conducted in three villages of district Kasur in Pakistani Punjab (Fox et al., 1985). The study (RCT) was based on post 1973 WHO protocols to investigate the susceptibility of *Plasmodium falciparum* to chloroquine. This randomized control trial observed 80% chloroquine sensitivity and 20% anti-malarial resistance. The RI resistance was 15% and RII resistance was 5%. It’s interesting that there was no evidence of RIII anti-malarial resistance during 1980s in Pakistan (Fox et al., 1985).

Rana et al (2004) estimated 13.11% incidence rate of malaria in five districts of Punjab; *P. vivax* was 4.08% and *P. falciparum* 9.03%. Moreover reported gender difference regarding incidence of malaria parasite; more male (53.5%) participants were affected than female (46.94%). There was significant resistance of RI, 35.1% and RII resistance was only 5.4%. A distinguishing discovery was the significant number of children cases regarding anti-malarial resistant in low transmission areas. The children age group 1-5 year had maximum RI and RII resistance (maximum chloroquine resistance was noted in the 1-5 year age group, e.g. RI, 41%; RII 8%). Thus primarily researchers are recommended to include children as a subgroup while testing anti-malarial efficacy in low transmission settings because children has high risk of treatment failure. Moreover study observed that incidence of *P. falciparum* is increasing compared with *P. vivax*. Furthermore this study also observed none of RIII cases in five districts of southern Punjab.

Shahani et al (2013) also observed gender difference regarding prevalence of anti-malarial resistance, male participants were found more effected than female. It could be due to exposure, because male spend more time outdoor than female in Pakistani context due to social structure, thus male gender are on high risk to mosquito bite than female. In this study chloroquine sensitivity was estimated 50% and RI resistance was 33.7% and RII 18.7%. Both RI and RII resistance together was 52.4%, findings support the argument that anti-malarial resistance trend is increasing because in1984 both RI and RII resistance was 20% and now it’s more than double. Furthermore, none of RIII case found regarding chloroquine resistance.

In 1999, Rawland et al found higher rates of chloroquine resistance than expected, thus study concluded that CQ is not suitable for first-line falciparum treatment among Afghan refugees in Pakistan. Later it was considered that extended dose of chloroquine could overcome about 39% of resistant infections, which could recrudesce under the standard regimen. But direct observation found high collapse rates; thus chloroquine is not an effective drug. The population movement could be another major factor for anti-malarial resistance among refugees because mostly they visit malaria endemic zones in Afghanistan.

Howard et al (2011) regarding dosage of chloroquine has been found higher parasitic recrudescence (84%) among those took CQ25 dosage than those took CQ40 dosage (51%). The cure rates were significantly improved with CQ40 treatment, particularly among adult participants. The clearance time of pyretic, clearance time of parasite and the proportion of gameto-cytaemic post treatment was parallel in entire treatment groups. Furthermore second-line CQ40 treatment resulted in higher failure rates than first-line CQ40 treatment. The CQ-resistance marker pfcrt 76T was found in all analyzed isolates. In addition pfmdr1 86Y was 18% and 184Y was 37% among analyzed isolates. This study was conducted in Afghan refugee camps, thus travel to endemic zones, low standard medicine and
education level of participants are main factors regarding anti-malarial resistance.

**Therapeutic Efficacy Tests Involving Other Acts (Based On Post 1973 WHO Protocols)**

The SP is not in use anymore as a monotherapy for *P. falciparum* but still in practice as a combination therapy (1st line treatment) with artesunate in many countries. For usefulness of combination therapy it’s important to monitor closely efficacy of SP component. In 1997, Rowland et al investigated resistance of *P. falciparum* malaria to chloroquine and sulfadoxine-pyrimethamine among Afghan refugees in western Pakistan. It was a first research that reported resistance of *P. falciparum* to chloroquine and sulfadoxine-pyrimethamine among refugees in Pakistan. Previously resistance to sulfadoxine pyrimethamine never been reported neither among refugees nor natives in Pakistan. The SP resistance could be transmitted through male Afghan refugees who often travel into Afghanistan because SP resistance is already reported in Afghanistan. Refugee villages are usually next to Pakistani villages and transmission has been shown to occur locally (Rowland et al., 1999). Shah et al (1997) also reported *P. falciparum* resistance to chloroquine in local Pakistani villages. Pakistan shares border with Iran in malaria endemic zone and drug resistance is prevalent in Iran (Shah et al., 1997).

In 1993, Khan et al recruited 100 patients with positive asexual *P. falciparum* and 60 patients were treated with quinine, then 13 of them developed complications. Another 2 were hypoglasimic, and 1 was with encephalopathy. The chloroquine drug was given to 15 patients and 11 were recovered but 3 were died and 1 patient found RI resistance and those 3 died had R1 and RI resistance but RIII resistance was not found. Another 15 patients treated with Halofantrion and 10 of them treated with fansidar, thus recovery rate was 100%. This study concluded that Halofantrion is observed most rapidly acting drug.

Rana et al (2011) found *P. falciparum* resistance to chloroquine and highest rate were observed among male gender (79.5%) than female (20.4%). A significant finding was highest anti-malarial resistance 31.8% among children (6-15 years). Similar trend was observed regarding basoquine resistance, more in male (72%) gender than female (28%) and highest resistance of 41.7% was among age group 6-15 years. This study observed that sulphadoxine-pyrimethamine was highly effective and resistance was only 5.7%. Overall resistance trend was similar among both genders and age groups regarding chloroquine and basoquine use. Furthermore, significant differences were found regarding sulfadoxine-pyrimethamine and chloroquine/basoquine use but regarding chloroquine and basoquine use there were no significant differences during statistical analysis.

During regression analysis chloroquine was found less effective than sulfadoxine-pyrimethamine and basoquine, odd ratios were 6.4 and 8.4 respectively. Conversely chloroquine and sulfadoxine-pyrimethamine were found equivalent in terms of efficacy, odd ratio was 1.3 (Rana et al, 2011). Thus significant finding was high incidence rate among children (6-15 years) and male gender were more affected than female because nature of job, poverty among children, poor treatment, diagnostic issues and lifestyle.

**Threat of Emergence of Artemisinin Resistance**

Progressive artemisinin resistance *P. falciparum* malaria has been reported during routine surveillance in Cambodia and Thailand (World Health Report, 2002 and WHO, 2004). Previously chloroquine resistance and sulfadoxine pyrimethamine resistance has been observed in Cambodia. Parasite clearance delay has been found during monotherapy (artesunate with mefloquine) treatment failure. Number of factors take part in emergence of decreased artemisinin sensitivity and one major factor is low standard tablets which contain poor action ingredients. In addition sub-therapeutic dosing is playing key role regarding selection of resistance parasite strains. Moreover other factors could be duration (artesininisin is in use from last 30 years), unique massive drug pressure, low malaria transmission, unique *P. falciparum* phenotype and host factors (Noedl et al., 2009; Dondorp et al., 2010). Artemisinin resistance was never considered important issue because it has
been effectively in use from centuries in China. Recently researchers observed limited resistance to artemisinin but it could be a major challenge to eradicate malaria worldwide because no alternative is available to date (Dondorp et al., 2009). WHO recently launched a multifaceted containment programme, called Global Plan for Artemisinin Resistance Containment (GPARC), which is based on prevention, early detection and elimination of resistance regarding artemisinin. In addition prevent artemisinin resistance in resistance free places (GPARC, 2011). Since 2008 artemisinin-based combination therapy (ACT) with sulphadoxine-pyrimethamine is first line treatment for *P. falciparum* in Pakistan. The researchers found 56% treatment failure of sulphadoxine-pyrimethamine monotherapy in Baluchistan province of Pakistan during 2001-05. Thus use of standard protocol invivo efficacy study of ACT through performing regular rounds is necessary. Incase within 72 hours >10% cases found positive then it should be regarded as a sign for further research. The ACT treatment failure shouldn’t be considered artemisinin resistance and additional study should be conducted with ART monotherapy for seven days to confirm ART resistance. The parameters should be parasite positivity at day 3 after treatment, pharmacokinetic measurements, parasite clearance time, and parasite reduction ratio within 48 hours and slope of linear parasite clearance curve (Noedl et al., 2009).

**LIMITATIONS**

It was not possible to give a perfect image of drug resistance malaria in the country because lack of published scientific information on topic in Pakistan but it was possible to give general overview on anti-malarial drug resistance in Pakistan. The major issue was inaccessibility of scientific articles on topic due to lack of research studies and publications in international scientific data basis. In addition, the language of publication other than English and free access to scientific articles were barriers. The University of Oulu student account was used to access scientific material, however not all material were retrievable.

**CONCLUSION**

Despite progress in health system malaria still remains a major burden on public health. In year 2001 Malaria Control Program, Ministry of Health Pakistan and World Health Organization planned to roll back malaria and bring malaria annual cases to 0.5/1000 till 2010 but proposed target became difficult to achieved (Rana et al., 2004). The major obstacle to roll back malaria in Pakistan is anti-malarial resistance and poor cure rates of anti-malarial drugs. The prevalence trend of *P. falciparum* has shown continuous increase in all endemic areas of Pakistan. Malaria manifestations are severely affecting societies and causing significant morbidity and mortality because malaria parasite continuously escapes from invader drugs and change shape for survival. Malaria infection creates huge economic loss and it become difficult for health system to properly allocate funds for primary health care in developing countries. Human health is primarily at stake due to malaria infection and major manifestations are jaundice, tachypnoea and hepatomegaly. If malaria infected patients do not get on time treatment then they develop complication as cerebral malaria, anemia, hemoglobinuria, pulmonary edema, cardiovascular collapse, shock, kidney failure, thrombocytopenia and hypoglycemia (Borosk, 2000).

Number of factors take part in emergence of decreased anti-malarial sensitivity and one major factor is substandard tablets which contain poor action ingredients. In addition sub-therapeutic dosing is playing worse role regarding drug resistance malaria. In some regions other factors could be duration, artemisinins used from last few decades. In addition unique massive drug pressure and low malaria transmission are facilitating anti-malarial sensitivity and resistance (Dondorp et al., 2010). Late diagnosis, lifestyle, male gender, traveling, poor diagnosis, vector control, education of medical practitioners and miss use of medicine are other significant factors for prevalence of drug resistance malaria in Pakistan. Most of studies observed that children are more vulnerable to anti-malarial resistance (6-15 years age group), in addition male gender were more affected, which could be due to nature of their work.
because Pakistan’s economy is agriculture based and male is responsible for livelihood of family, therefore most of agriculture and other jobs are outdoor and male are on high risk to mosquito bite than female. In Pakistani context encouragement of early diagnosis, proper treatment, reduce drug pressure, optimize vector control, target mobile population, proper management, good surveillance and operational research is important. In addition researchers are recommended that to include children as a subgroup whenever testing anti-malarial efficacy in low transmission settings because in children risk of treatment failure is high. This study found low number of published invivo studies regarding anti-malarial resistance in Pakistan, thus there is a pressing need of more studies to observe, find obstacles and conduct ant-malarial resistance surveillance in Pakistan. Drug efficacy trials conducted in Pakistan has given important indication to guide country’s national malaria treatment policy. Thus in-depth observation of current first line malaria treatment in Pakistan is necessary. It enhance concerns since SP is first line malaria treatment drug with artemisinin and it would lead to failure of ACT treatment. In addition it exposes artesunate to drug pressure which leads to surfacing artemisinins resistance. Thus there is need to monitor response of anti-malarial and take measures to prevent surfacing of anti-malarial resistance. Prevention of resistance could be possible through access of high quality effective ACTs on large scale. Furthermore there is need of proper education of practitioners, raise observance of ACTs users, proper diagnosis to avoid medicine misuse, transmission control to decrease use of anti-malarial drugs and diminish reservoir of infection.

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