Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/7523
DOI URL: http://dx.doi.org/10.21474/IJAR01/7523

RESEARCH ARTICLE

DESCRIPTION OF HAEMATOLOGICAL TREND OF VIVAX MALARIA TREATED WITH CHLOROQUINE AND PRIMAQUINE IN EASTERN AFGHANISTAN LAGHMAN PROVINCE.

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Manuscript Info

Manuscript History

Received: 06 June 2018
Final Accepted: 08 July 2018
Published: August 2018

Abstract

Background: Malaria is a major cause of morbidity and recognized as a heavy burden on health system in Afghanistan.,95% of confirmed cases are due to P. vivax which is challenging because of the non-specific nature of the signs and symptoms, relapses of the infection weeks to months after the initial attack for up to about 2 years and requires radical treatment but still not well-administered due to fear of hemolysis as according the literature the Mediterranean variant of G6PD is common in many ethnic groups in Afghanistan, Primary objective**:** To determine the normal hematological response, following *P. vivax* infection and treatment in malaria endemic population in Afghanistan.

Secondary objectives: 1) To measure the hemoglobin difference before and after treatment. 2)To assess independent risk factors associated with anaemia. 3)To assess the time to recovery from anaemia after administration of chloroquine+primaquine and compare this with chloroquine alone 4) To assess the effect of primaquine mg/kg dose on hemoglobin reduction and time to anaemia recovery. 5) To describe evidence of hemolysis in patients receiving primaquine. 6) To estimate the prevalence of G6PD deficiency.

Method: This was a single-arm observational cohort study for descriptive prospective analysis of clinical, Laboratory, and demographic data from outpatients with P. vivax malaria. Those who had parasitological confirmed *Plasmodium vivax* with thick blood smear examination who were meeting study inclusion criteria, gave consent and enrolled in the study, had their G6PD status, CBC including the WBC count, hemoglobin values, and platelets counts performed and after a careful clinical assessment the treatment prescribed according to the malaria treatment protocol (14).

Results: Overall 221 patients have been recorded, however, the target were 250 patients but, 29 of them were recorded as defaulters. Adherence to 14 days PQ therapy in G6PD normal patients were excellent (100%), all patient in intervention group fully completed 14 days PQ therapy.

Conclusion: Anti-relapse therapy is recommended for all confirmed PV cases. In the current situation, G6PD RDT can be used at lower health facilities.

Acknowledgements: I as the author of this study, express my gratitude to my supervisors at Department of Public Health, Maulana Azad

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University, Dr Latika Nath Sinha, Ms. Bhawana Sathi, Dr Abhishek Lohra, Dr Nitin Joshi and Dr Pr. Amila Vivek and all guest professors who taught the conduct of research and provided their valuable comments while writing up this paper. Likewise, I acknowledge the support of my co-supervisor, Dr. Ghulam Rahim Awab, MD, MPH Lecturer of Nangarhar university of Afghanistan for his support during complete course of MPH program, reviewing the report and valuable assistance.

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Abbreviations:-

CHC	Comperhensive Health Center
BHC	Basic Health Center
F	Female
M	Male
Y	Year
RR	Respiratory Rste
PR	Pulse Rate
BP	Blood Presure
G6PD	Glucose- 6 Phosphatase Dehydrogenase
G6PDd	Glucose 6 Phosphattase Dehydrogenase deficincy
CQ	Chloroquine
PQ	Primaquine
CRF	Case Record Form
BHC	Basic Package of Health Services
EPHS	Essential Package of Health Services
RDT	Rapid Diagnostic Test
CBC	Complete Blood Count
NMLCP	National Malaria and Leishmaniosis Control Program
SHC	Sub Health Center
MHT	Mobile Health Team
CBMM	Community Based Management of Malaria
NTG	National Treatment Guideline

Introduction:-

Despite the progress in reducing its morbidity and mortality, malaria still remains a major public health problem in many countries of the world, including Afghanistan and its elimination is one of the greatest current global health challenges. Achieving elimination in practice requires implementation of control measures across a wide range of health care settings. There is increasing evidence that P. vivax results in significant morbidity and associated mortality and is more difficult to eliminate than P. falciparum because of relapse from dormant liver hypnozoites, sustaining infection across transmission seasons. Plasmodium vivax is responsible for more than 90% of laboratory-confirmed malaria cases in Afghanistan and continues to cause considerable morbidity in several areas of the country.

A significant proportion of the above morbidity and low-birth-weight (LBW) infants of infected mothers is attributable to chronic or severe anaemia from recurrent P. vivax infections, where each relapse results in an increased cumulative risk of anemia. Malaria-associated anemia is a complex phenomenon, related to increased red cell destruction, dis erythropoiesis and haemopoeitic suppression, compounded by nutritional status, helminthes carriage and drug induced hemolysis. The degree of anemia caused by vivax malaria and its risk factors has not been evaluated widely [2].

For P. vivax malaria specifically, antimalarial treatment and anemia are inextricably connected. Radical cure requires the use of a hypnozoitocidal agent, of which the only one widely available is primaquine [3]. Intravascular hemolysis occurs in patients whose erythrocytes are congenitally deficient in G6PD enzyme following oxidative

stress triggered by drugs (such as primaquine, dapsone, chloramphenicol and ciprofloxacin), infection, or the ingestion of fava beans. G6PD deficiency (G6PDd) is also associated with an increased risk of neonatal jaundice, and rarely with chronic non- spherocytic haemolytic anemia and gallstones. The degree of enzyme deficiency and severity of clinical complications depend on the exact G6PD mutation involved. The Mediterranean and certain Southeast Asian variants are associated with less than 10% residual enzyme activity (Class II deficiency) [4] and generally more severe clinical manifestations than the African A- form, which provides 10–60% of residual activity (Class III). Since the G6PD gene is found on the X-chromosome, there is a higher risk of haemolytic crisis in males (homozygous) and homozygous females with mutations than in heterozygous females, although heterozygotes are also at some risk due to X-chromosome inactivation [5].

The term 'Mediterranean' is used to describe the 563C.T mutation in exon 6 of the human G6PD gene (changing a serine to a phenylalanine residue at position 188 of the protein product), reflecting the first description of this variant in countries such as Italy and Cyprus, in many other Middle Eastern countries and the Indian subcontinent and has been documented as Far East as China [6], Malaysia [7] and Singapore [8]. In south Asia this variant of G6PDd is of practical relevance as there is substantial geographical overlap with malaria. The administration of primaquine to clear the hypnozoit forms of P. vivax [9], is compromised by the lack of readily available tests to exclude G6PD deficient individuals in Afghanistan, resulted in foremost impediment of preventing recurrent disease episodes and failure of policy to be translated to practice, representing a major obstacle to malaria control efforts in Afghanistan and the wider region.

Control and ultimate elimination of P. vivax requires the safe and effective cure targeting both blood and liver stages of the parasite. In Afghanistan, chloroquine remains first-line treatment for the erythrocyte stage of P. vivax infection, with no evidence of resistance, although it provides a shorter period of post-treatment prophylaxis than DHA-primaquine in a previous comparative trial (10). Limited resources and security challenges hamper malaria control efforts (11), In order to quantify the risks and benefits of P. vivax current treatment, it is crucial to determine the normal hematological response following P. vivax infection and treatment. The Complete Blood Count (CBC) assesses the cellular elements of the blood, that is, red cells, white cells, and platelets, both qualitatively and quantitatively (12) and is an essential tool in assessing hematological changes. The leukogram is part of the CBC that analyzes white blood cells; it comprises the total WBC and subpopulation counts including neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Their reference values vary according to age, race, physiological condition (pregnancy), use of some drugs, and time of day (13). We also aim to investigate G6PD activity particularly, in heterozygous females in order to explore the diverse mode of the inherited enzymopathy in Afghan population. Our purpose is to encourage early and prompt treatment of patients at risk of complications and improve their disease outcome.

Most fever in Afghanistan not caused by malaria. In case of confirmed malaria, the treatment for these two species (Pf and Pv) are differs, therefore identification of these species is important for treatment outcomes. The main quality-of-care challenge posed by the recent decline in malaria is now to identifying those cases of fever that, are in fact caused by malaria amongst the clinical malaria cases and treating the correct species of infection according to National Treatment Guideline (NTG).

There are presently many efforts to improve and enable community level for malaria control within an expanding program in country. The Government of Afghanistan remains committed to the control of this disease. For this purpose, for managing of malaria cases among fever cases at community level, the malaria control administration developed Community-based Management of Malaria Strategic Plan (CBMM) and has been started deployment of malaria Rapid Diagnostic Tests (RDTs) through Basic Package of Health Services (BPHS) implementers, since 2013.

Review of Literature:-

Literature Review was conducted through researching related site in the internet, NMLCP and partner published and unpublished document and malaria journal, I found several related researches conducted in Afghanistan and region which shows prevalence of G6PD Deficiency among Afghan population, side effect of primaquine in G6PD Deficient person and rule of primaquine in radical treatment of Pv cases.

Considering studies conducted in the field of G6PD, my study will provide useful information for National Program regarding feasibility and accessibility of G6PD test for vivax cases under the current health system structure.

Malaria in Afghanistan is typical of most of Southern and Western Asia and can be summarized as hypo-endemic unstable transmission. Transmission is seasonally and geographically constrained with mixed endemicity. Plasmodium vivax predominates, accounting for 95% of cases (2016 NMLCP Report) and an incidence of approximately 10-100 per thousand per year in the most endemic areas. The remainder of cases are caused by P. falciparum with approximate annual incidence of <1-10 per thousand per year (Rowland et al. 1994, Rowland et al. 1999, unpublished clinic data).

P. vivax incidence varies throughout the year, with a peak in the summer months, generally assumed to be caused by relapses from infections transmitted in the previous year. P. falciparum cases are seen over the late summer and autumn, usually from August to December (Fox & Strickland, 1989, Rowland et al 2002). Geographically, malaria is confined to areas below 2000m above sea level and is limited by environmental and human factors (Brooker et al, 2006).

The majority of malaria episodes are caused by P. vivax infections. The ability of this species to form hypnozoits in the liver contributes to its stability. Even with effective treatment for acute episodes, one initial infection may lead to up to 10 or more subsequent episodes (Leslie et al, 2004; Leslie et al, 2008). Not only does this cause excess morbidity, but also acts as a reservoir of disease. It is this reservoir which allows the disease to continue its transmission cycle. This ability to produce a latent liver stage also reduces the efficiency of insecticide treated nets as a control tool; infected persons may develop further episodes, even if they regularly use an ITN (Rowland et al, ITN Paper).

CQ still remains highly effective against vivax malaria (Leslie et al 2004, Leslie et al, 2007, Leslie et al, 2008) Awab 2011, 2014 and NMLCP 2016, despite reports of resistance from other parts of Asia (REF). However, the radical cure of vivax malaria, using primaquine or other anti-hynozoite drugs, still presents considerable challenges. Although effective when given as the standard 14-day course, and a recently tested 8-week regimen, primaquine cannot be readily used in the population (Leslie et al. 2004; Leslie et al 2008). The high prevalence of the common enzymopathy glucose 6 phosphate dehydrogenase (G6PD) deficiency (Bouma et al, 1995; Ali et al, 2007) precludes its use because, in the presence of primaquine, G6PD deficiency can cause clinically significant and occasionally severe haemolytic anemia (Bouma et al 1995). Since G6PD testing is not readily available, anti-relapse therapy cannot be made widely accessible to most populations at risk of vivax malaria. The risk to benefit ratio is not seen as favorable.

The most recent data available (HN-TPO, 2008) shows that G6PD testing is not readily available at clinics and major health sector in the public or in the private sector in Afghanistan. The same study also showed that most patients who have a fever in endemic areas seek treatment (>90%). Thus, most cases are treated in the formal health sector (either public or private sector). With the broadening of access to diagnosis (i.e. implementation of rapid diagnostic tests and microscopy) increased numbers of patients will have confirmed vivax diagnoses. This presents an opportunity to simultaneously increase access to G6PD testing and therefore to anti-relapse therapy with primaquine.

We propose to conduct an operational research project to test the effectiveness of a referral and treatment system for vivax malaria. Under the system, all patients in the study area who are positive for vivax malaria will be referred for G6PD testing at a local laboratory acting as a central point for testing in the district. If they are confirmed to be G6PD non-deficient, they will be administered primaquine.

A. ***Research Question/Hypothesis:***

Can referral system work for vivax cases under current health system in Afghanistan?
Can program provide G6PD testing facility for radically treatment of vivax cases by administering primaquine 14 days course?

Objectives of the study:-

Primary objective: To determine the normal hematological response, following *P. vivax* infection and treatment in malaria endemic population in Afghanistan.

Secondary objectives:

1. To measure the hemoglobin difference before and after treatment of vivax malaria.
2. To assess independent risk factors associated with anemia at the start of treatment for vivax malaria.
3. To assess the time to recovery from anemia after administration of chloroquine+primaquine and compare this with chloroquine alone treatment.
4. To assess the effect of primaquine mg/kg dose on hemoglobin reduction and time to anemia recovery.
5. To describe If there is any evidence of hemolysis in patients receiving primaquine, particularly female heterozygotes with a normal rapid test result.
6. To estimate the prevalence of G6PD deficiency in vivax affected human population.

Methodology:-

Study Design:

1. Two districts (Mehterlam and Qarghaiee) of Laghman Province were selected. This was a single-arm observational cohort study for descriptive prospective analysis of clinical, Laboratory, and demographic data from outpatients with P. vivax malaria, from endemic province Laghman in Afghanistan. Patients Participants with suspected malaria symptoms have been initially screened those who had parasitological confirmed *Plasmodium vivax* with thick blood smear examination who were meeting study inclusion criteria, given consent and enrolled in the study ,and their G6PD status measured by the Care-Start G6DP rapid diagnostic test (G6PD RDT), complete blood counts including the WBC count, hemoglobin values, and platelets counts performed and after a careful clinical assessment the treatment prescribed according to the malaria treatment protocol (14). According to the G6PD RDT result, chloroquine prescribed at 10mg/kg at first two days and 5mg/kg as third daily dose. primaquine prescribed at 0.25mg/kg/day for 14 days (normal patients) or 0.75mg/kg weekly for eight weeks (deficient patients) (15).

They give information (verbal, written and pictorial) on the concept of G6PD deficiency and vivax malaria. The information emphasizes the importance of G6PD testing and anti-relapse therapy in prevention of further episodes of malaria. It provides information on the location of the G6PD testing center, that the testing is free and that it is strongly recommended to attend the center. Their contact details noted and they asked their location in the study area for later follow-up.

Patients are reviewed at day 2, day 7 and day 14, day 21 and day 28. At these visits patients undergone a brief clinical assessment and a small blood sample taken for repeat CBC measurement and dried blood spot for carboxy primaquine measurement (day 7 and day 14 only).

In general, antimalarial treatment unsupervised to reflect field conditions. Sufficient primaquine given to last until the next visit. From our own work and that of others in a similar population, adherence in this population was good, but this was checked by pharmacological assessments of carboxy primaquine levels.

At the end of the follow-up period, patient data was entered in to Excel sheet.

The primary outcome of the study was the proportion of patients who experience a second episode of vivax malaria during the period of observation (14 days post treatment). Secondary outcomes include the number of further episodes, relative rate (incidence) of disease (time series analysis). Amongst the intervention group the proportion referring (response rate), proportion receiving the G6PD test (test rate), the proportion receiving the first dose of treatment (treatment rate) and proportion completing treatment (completion rate) calculated.

B. ***Study area/setting:***

The study was conducted in two selected districts of the Laghman Province of Afghanistan. The area was characterized by seasonal transmission of predominantly vivax malaria and is typical of endemic areas in the country. In 2016, 14971 cases of vivax and 655 cases of falciparum and 10611 of clinical malaria were reported from health facilities of Laghman. The province has a developing health system which replicates the Basic Package of Health Services and EPHS, the centrally proscribed public health system. The system has a well-defined pyramidal structure with Health Posts at community level manned by community health workers; Basic Health Centers (BHC) staffed by at least one qualified practitioners; Comprehensive Health Centers (CHC) Malaria diagnosis is available at CHC level and above, where laboratories are available. Some BHCs also have microscopy available. Use of rapid diagnostic tests is Widely started under Global Fund project in community (Health Post) and

lower health facility without microscopy (SHC, MHT and BHC) in 2016. All BHCs in the selected districts were included in the study until the required sample size was reached.

C. ***Study subjects:***

Patients in the study districts who present at clinics which provide malaria microscopy was enrolled in the study when they give informed consent and meet the inclusion and none of the exclusion criteria.

I. Eligibility (Inclusion and Exclusion) Criteria

The following patients have been included:

Inclusion Criteria:

- Fever or history of fever in last 24 hours
- Laboratory diagnosed with *P. vivax* attending the study site
- Providing written informed consent
- Age ≥ 6 years
- Ability to swallow oral medication
- Ability to attend follow up visits

Exclusion Criteria:

- Refusal to participate
- Blood transfusion within the last 12 weeks
- Other infectious or Severe diseases
- Pregnancy or lactation
- History of hypersensitivity (allergy) to primaquine or chloroquine
- Inability to comply with follow-up visits

Sample size:

For the primary outcome (Hemoglobin recovery) the sample size calculation was based on the anticipated proportion of the vivax malaria patients with pre-treatment anemia assuming 45% and an anticipated proportion of patients remained anemic at Day28 about 20% (based on previous work) (16), with 25% lost to follow-up and uncompleted treatment carried out on 250 patients.

This was expected the sample size will give us 5 G6PD deficient individuals but, G6PD deficiency was zero in this research.

D. ***Sampling technique:***

All patients presenting at clinics in the study districts who meet the inclusion and none of the exclusion criteria were enrolled in the study until the required sample size was reached.

E. ***Data Collection methods, instruments used and measurements:***

The study used three principle data collection tools. The patient enrollment form was used at the Comprehensive Health Centers and Basic Health Center and was noted the patient's personal information and other key variables to be examined. At the CHCs and BHC laboratory, a laboratory record was held. This was noted the date of the test, the G6PD result and whether or not the patient received the first dose of primaquine. A patient follow-up form was used by CHWs responsible for the follow-up of the patients. This was noted information on further episodes of malaria, of adverse events and of completion of treatment. Episodes of malaria presenting at the clinics were recorded on the patient enrollment form. All forms and records was linked by patient number which was assigned on enrolment using pre-printed labels applied to all forms.

Key variables examined in this study was:

- Primary outcome: Episode of further slide confirmed malaria.
- Potential confounding factors include (but at this stage are not limited to): Age, sex, district (control or intervention), socio-economic status, ethnic group, dates of additional episodes (time to failure), village, background estimated ITN coverage, local level of transmission (estimated using clinic data).
- For the economic analysis, we used cost ingredients including (but at this stage not limited to): Health Service Costs (direct costs of supplies and reagents, drugs, human resource costs, discounted equipment costs) and Patient Costs. This data collected at the initial consultation and at the laboratory.

F. ***Techniques used:***

Malaria microscopy was conducted in the clinic by trained microscopists. Slides was fixed with methanol, air-dried and geimsa stained with 10% geimsa using thick and thin blood films. Slides were stored for later confirmation.
The G6PD test was done at lab using fluorescence test and the result (Normal and Deficient) was regarding in Lab register.

The quality control measures and good practices followed during the study implementation.

All health facilities Lab Technicians were received refresher training prior to implementation of the study to ensure accuracy at the primary clinics. All blood smears were double read at a central reference laboratory (NMLCP) by experienced microscopy trainers.

The G6PD test was conducted by trained technicians using standard operating procedures which have been used in country.

G. ***Study definitions (case definition):***

Case: History of fever with slide confirmed vivax malaria
Failure: Any episode of vivax malaria in the period of observation
Loss to follow-up: Unable to be contacted on two consecutive follow-up visits
Withdrawal: Withdrawn for adverse events, protocol violation or withdrawal of consent.

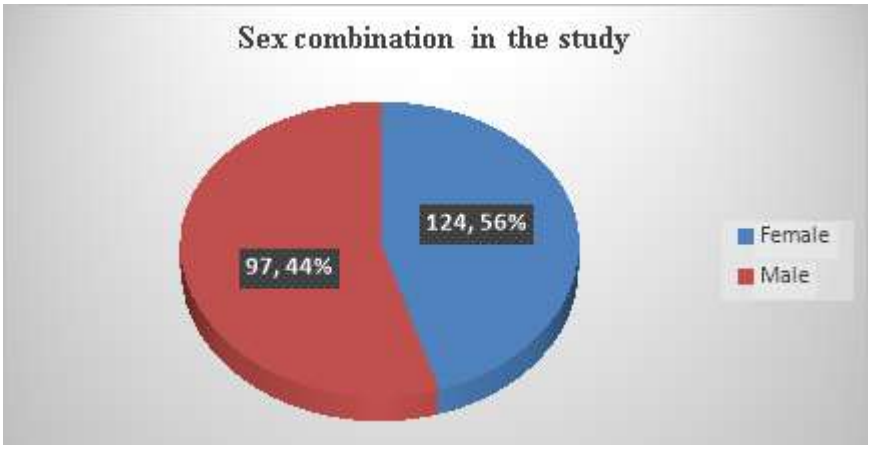
H. ***Data management and analysis plan:***

Data was recorded on pro-forma data collection forms. They were collected from the study sites at the end of the study. Data was e entered into E.

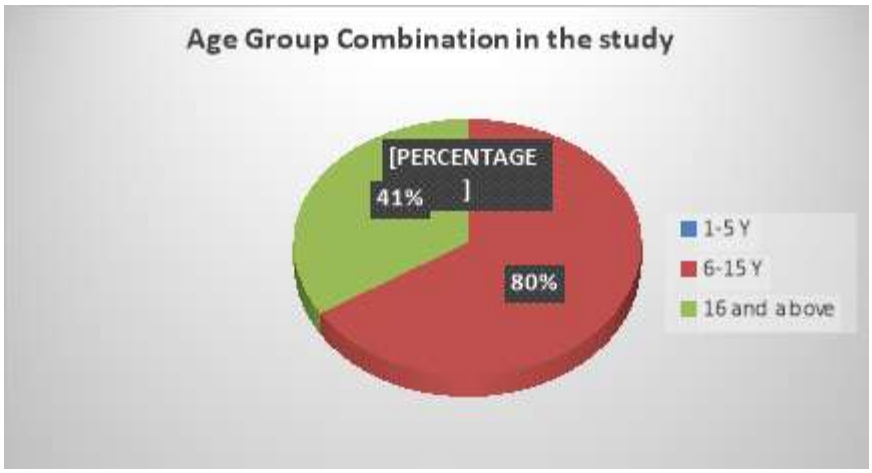
Results and Discussions:-

I. Characteristics of study participants:

The sample size of the study was 250 patients in planning phase 2:1 ration. The loss to follow-up rate was 9% (n-10 patients) in control district and 2% (n-4 patients) in intervention district. Analysis was done on 221 patients. The participant response was collected in pre-developed forms during study implementation and follow up. Data was double entering in Epi info 7. Also, Epi Info7 and excel were used for analysis.
Sex combination in the study was 97 (44%) male and 124 (56%) Female.

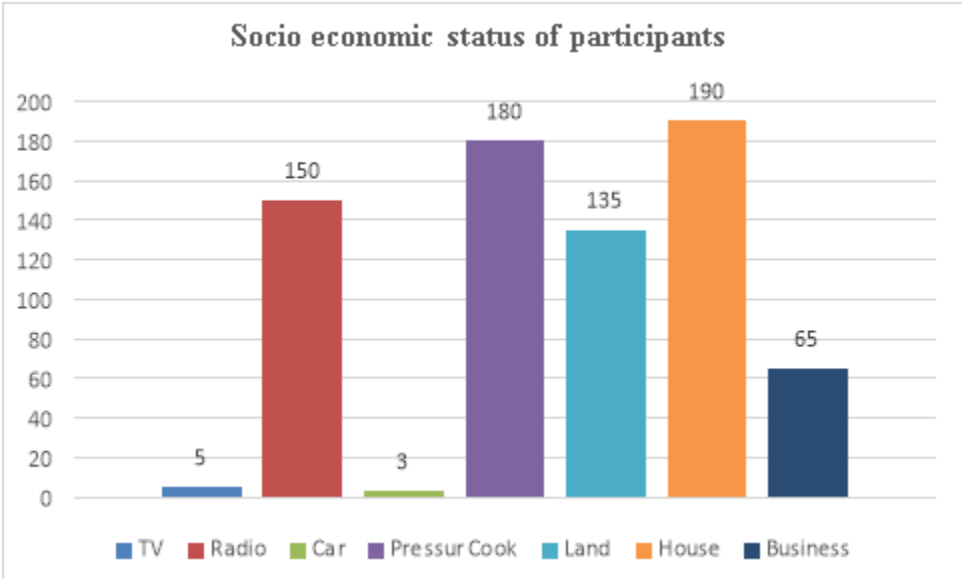


Age group combination in both districts was near to each other, smallest age was 6 and oldest age was 52-year-old



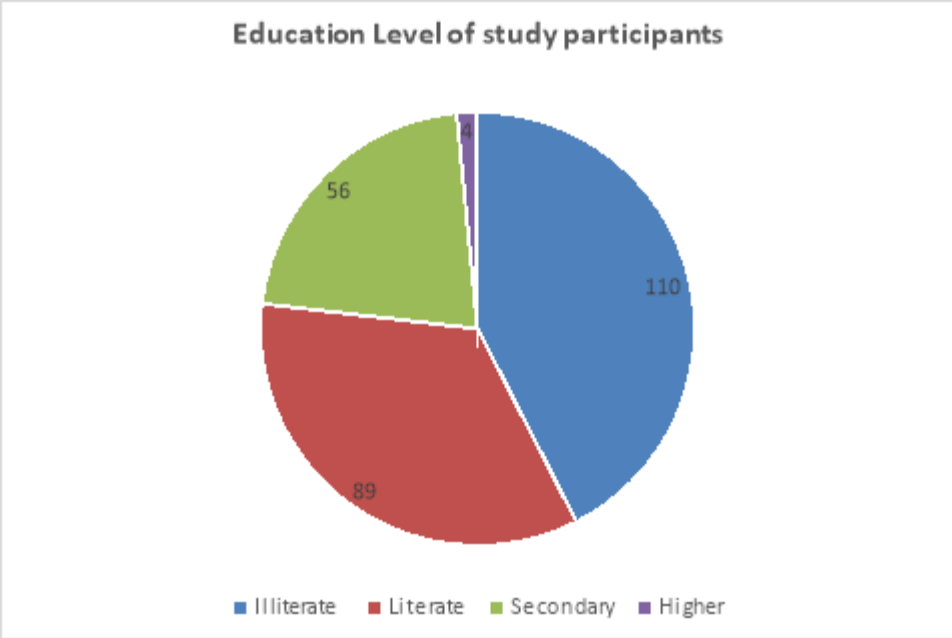
J. ***Socio economic status of study participants:***

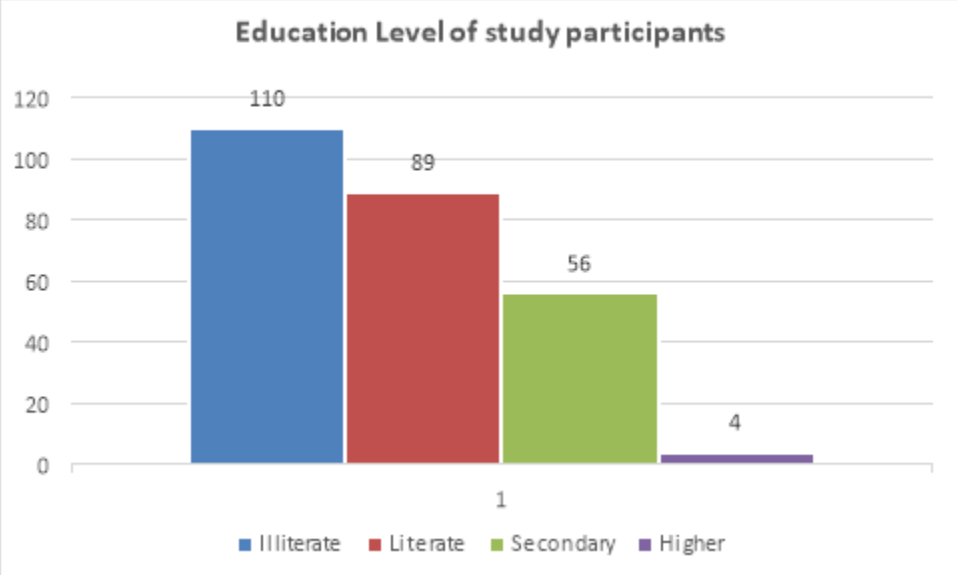
Socio economic status of study participant were check by asking study participant about the following:



K. Education level of study participants:

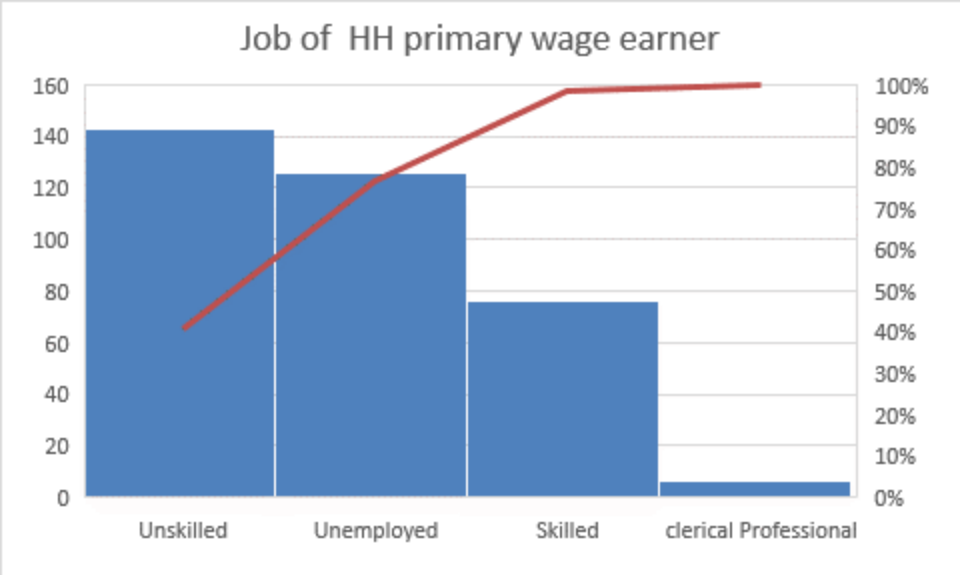
Overall education level of study patients was low, only 4 patient had higher education 56 patients with secondary education. Literate 89 and 110 Illiterate.





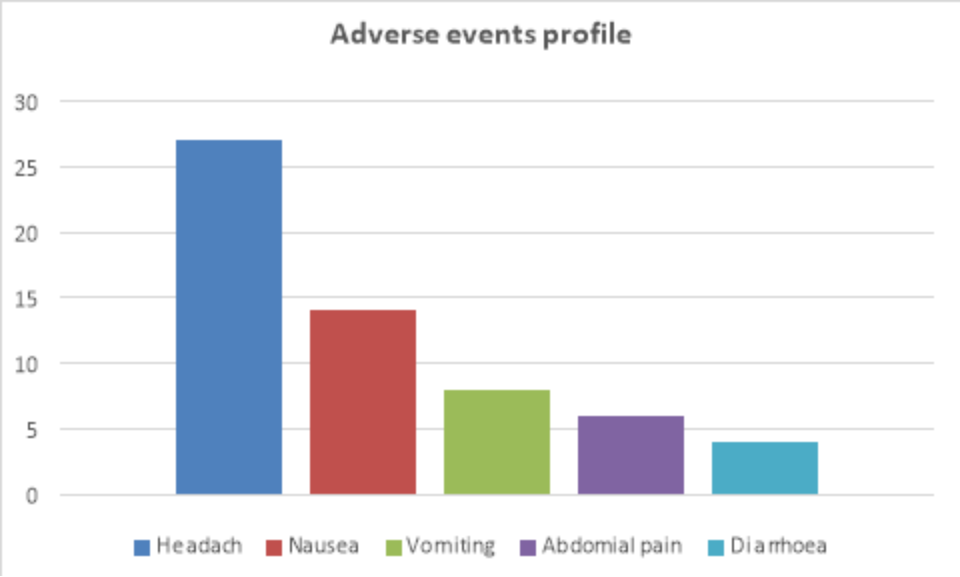
L. ***Job of House Hold primary wage earner:***

The majority of house hold primary wage earner was unemployed (n-126), the lowest score was 6 for clerical professional, showed in below graph:



M. ***Adverse events Profile:***

The highest score for adverse events among study patient was for headache (n- 27, 12% of study patient complain from headache) and the lowest score was for diarrhea (n-4, 2% of study patient), there was no complain from cyanosis, Anemia, Dark colored urine, convulsion and skin disorder, showed in below graph.



N. ***Results against objective of study:***

1. To measure the hemoglobin difference before and after treatment of vivax malaria.
2. To assess independent risk factors associated with anemia at the start of treatment for vivax malaria.
3. To assess the time to recovery from anemia after administration of chloroquine+primaquine and compare this with chloroquine alone treatment.
4. To assess the effect of primaquine mg/kg dose on hemoglobin reduction and time to anemia recovery.
5. To describe If there is any evidence of hemolysis in patients receiving primaquine, particularly female heterozygotes with a normal rapid test result.
6. To estimate the prevalence of G6PD deficiency in vivax affected human population

G6PD test was conducted for each PV confirmed patient in intervention districts (n-221), all patients prescribed a 14-day course of primaquine (0.25mg/kg per day) after three days treatment with standard dose of chloroquine.

The effectiveness of field based G6PD testing and radical cure of vivax malaria on incidence of vivax malaria was one of the main objective of this study, there were no relapse cases in intervention group, but 9% relapse cases were reported from control group which shows the effect of primaquine in radical treatment of Malaria.

Adherence to 14-day PQ therapy in G6PD normal patients were excellent (100%), all patient in intervention group fully completed 14-day PQ therapy.

Primaquine daily dosing:
This dosing table provides a daily dose of between 0.19 and 0.33 mg/kg primaquine (total dose over 14 days 2.6 – 4.7 mg/kg). Children will be offered sweet drinks once the drug been administered to minimize the chance of vomiting. Parents of children will be shown how to measure the dose to allow accurate home administration.

Weight (kg)	Daily dose	Total dose received (mg/kg)
8 or less	1ml suspension*	2.6 - 4.2
9-14	2ml suspension*	3.0 – 4.7
15-20	3ml suspension*	3.2 – 4.2
21-26	4ml suspension*	3.2 – 4.0
27-36	Half tablet	2.9 – 3.9
37-52	Three-quarters tablet	3.0 – 4.3
53 or more	Whole tablet	2.6** - 4.0

Discussion:-

Malaria continues to be a major public health problem in Afghanistan and 95% of the malaria cases attributed to P.v. and balance 5% to P.F. Malaria transmission is seasonal.

27% of Afghan population lives in areas at high risk for malaria, 49% at medium risk and the remaining 24% live in areas with no risk or very low risk of malaria transmission.

There has been gradual increase in the number of confirmed malaria cases during 2014-2016 (83,920, 103,377 and 190,231 respectively) due to improved access to diagnosis through the expansion of CBMM, using RDT.

In 2016, 85% of confirmed P.V and 84% of P.F. cases were reported from 5 provinces - Nangarhar, Laghman, Kunar, Khost and Paktika. 89% of malaria deaths were reported from Nangarhar, Kabul and Kunar.

The genotypic study conducted in 2014 in 9 provinces of Afghanistan, confirmed the prevalence of G6PD deficiency in males with the highest frequencies in Pashtuns and Pashais (10%). To determine the normal hematological response following *P. vivax* infection and treatment.

One main reason to conduct this study was to determine the normal hematological response following *P. vivax* infection and treatment.

NTG, 2017 recommend primaquine for each confirmed vivax malaria cases, those who tested G6PD status and they are normal, 14 days treatment regime is recommended, those who are not tested, weekly dose for 8 weeks is recommended. Still some practitioners not prescribe primaquine, the main reason they said is the side effect of primaquine in G6PD deficient patient.
In this study, I found the level of G6PD deficiency rate very low 0.45% compare to previous study 10%, rate of referral system from lower health facility to G6PD testing center were 65.8% in control district and 82.8% in intervention districts. Considering the above result, I recommend NMLCP to thinks regarding below options:

1. Develop and implement referral system for G6PD test, NMLCP will need on the system to encourage patient to visit referral center for G6PD test, because current rate in control district is low (65.8%), it may be lower in normal situation.
2. Current study in the region show the effectiveness of G6PD RDT which is also best option in Afghanistan situation in lower health facility based on feasibility concern. NMLCP should think regarding G6PD RDT implementation in their policy, because expansion of fluorescence test to lower health facility is not look possible under current health system infrastructure.

3. Work closely with HF practitioners to encourage them on weekly dose of primaquine under close observation of patient, because G6PD deficiency rate is very low 0.45%

Conclusion and recommendation:-

1. Anti-relapse therapy is recommended for all confirmed PV cases, as more than 95% of malaria cases are due to PV in Afghanistan, also current strategy is <Malaria control toward elimination> which may not be achievable without radical treatment
2. In the current situation, referral system for G6PD will not be an excellent option, therefore it would be better to work on other option especially G6PD RDT which can be used at lower health facilities (HPs)
3. Although G6PD deficiency rate was low (0.45%) among the study population compare to other study in the region, but it should not be ignored, program should provide G6PD testing facility to all confirmed PV cases or should treat them with primaquine on weekly base under strong observation program.

On day 0

A total of 221 patients were examined in different HFs for different tests, the results is as follow:

Indicators	Age by Year	Height	Weight	Sex Ratio F/M	Vital signs					Parasitemia		HB result	TLC
					Temperature	RR	PR	BP Systolic	BP Diastolic	Trophozoitr	Gametocytes	gm/dl	Number
Mode	9	110	20	1.3	37	20	90	90	70	3200	1230	11	7400
Mean	15	135	35		37	20	96	97	69	8804	1186	11	6637
Median	10	134	29		37.5	20	92	95	70	4800	1230	11	6700

The results for differential diagnosis for leucocytes are as follow:

	DLC					Medication	
	N %	L%	B%	M%	E%	CQ	PQ
						Number of tab	Number of Tab
Mode	62	34	0	4	1	2	1
Mean	62	33	0	4	1	2	1
Median	62	33	0	4	1	2	1

In order to control the fever, 114 out of 221 patients were received Acetaminophen, 37 were received Brufen and the remaining patient were received HE and cold clam for reducing of fever.

On day 2nd, the following indicators/statistic shows the result of the 221 patients visited in different HFs:

In order to subside the anemia, the 36 out 221 patients were received Ferrous Sulfate Tabs but the HB was not considered.

The HB of 184 patients were less than 12 but 36 patients with different HBs received the Ferrous tabs and the HB of 49 patients
were less than 10 but 12 out of 49 were received Ferrous Tablets.

The following table shows the day 7 result of the patients:

The clinical status of all of them was ok and none of them was reported with fever and other symptoms except

Statistic/indicators	Vital signs					Paracetamol		HB result	TLC	DLC					Medication	
	Temperature	RR	PR	BP Systolic	BP Diastolic	Trophosoitr	Gametocytes	gm/dl	Number	N %	L%	B%	M%	E%	CQ	PQ
Mode	37	17	80	90	70	0	0	11	6400	62	33	0	4	1	0	1
Mean	37	17	77	99	72	0	0	11	6703	61	34	0	4	1	0	1
Median	37	17	76	90	70	0	0	11	6800	62	33	0	4	1	0	1

89 out of 221 patients were received Ferrous sulfate TBs and the remaining of them were received Health education anemia in some cases but the HB level was more than 10 as you may see it in above table

The result of day 14 is as follow:

Statistic/indicators	Vital signs					Parasitemia		HB result	TLC	DLC					Medication	
	Temperature	RR	PR	BP Systolic	BP Diastolic	Trophosoitr	Gametocytes	gm/dl	Number	N %	L%	B%	M%	E%	CQ	PQ
Mode	36.5	16	70	90	70	0	0	10.8	7100	62	33	0	4	1	0	1
Mean	36.5	16.9	75	99.7	72.2	0	0	11.5	6883	60.4	34.5	0.02	4.05	1.46	0	1.17
Median	36.5	17	75	90	70	0	0	11.4	7000	61	33	0	4	1	0	1

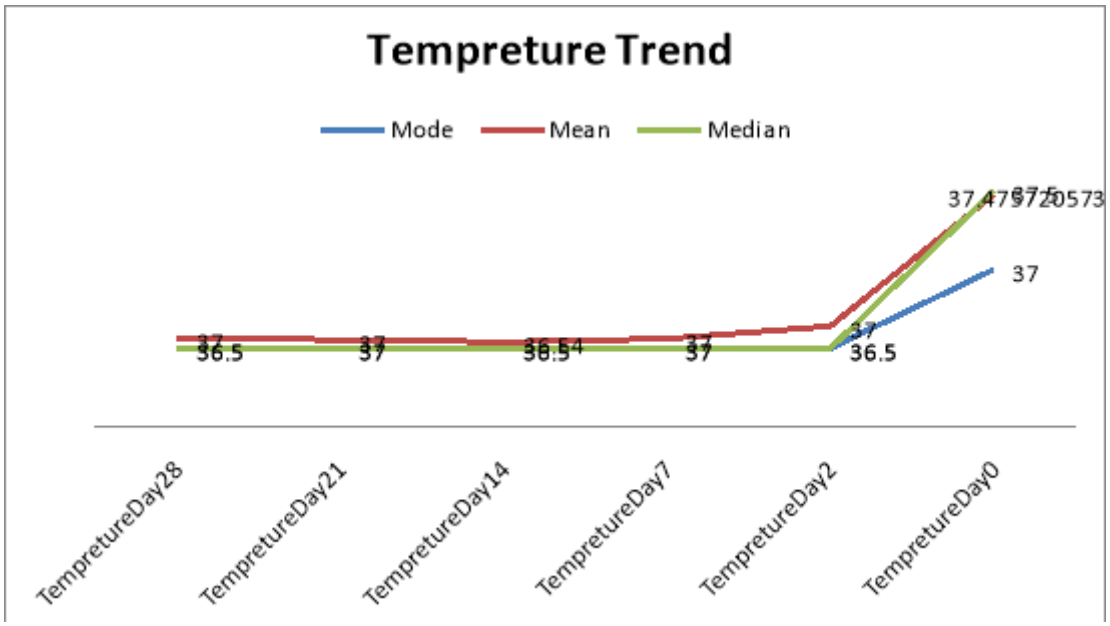
The result of day 21st is as follow:

Statistic/indicators	Vital signs					Parasitemia		HB result	TLC	DLC					Medication	
	Temperature	RR	PR	BP Systolic	BP Diastolic	Trophosoitr	Gametocytes	gm/dl	Number	N %	L%	B%	M%	E%	CQ	PQ
Mode	37	18	70	90	70	0	0	11	7000	58	37	0	4	1	0	0
Mean	37	19	73	99	72	0	0	12	7067	59	36	0	4	2	0	0
Median	37	19	71	90	70	0	0	12	7200	58	36	0	4	2	0	0

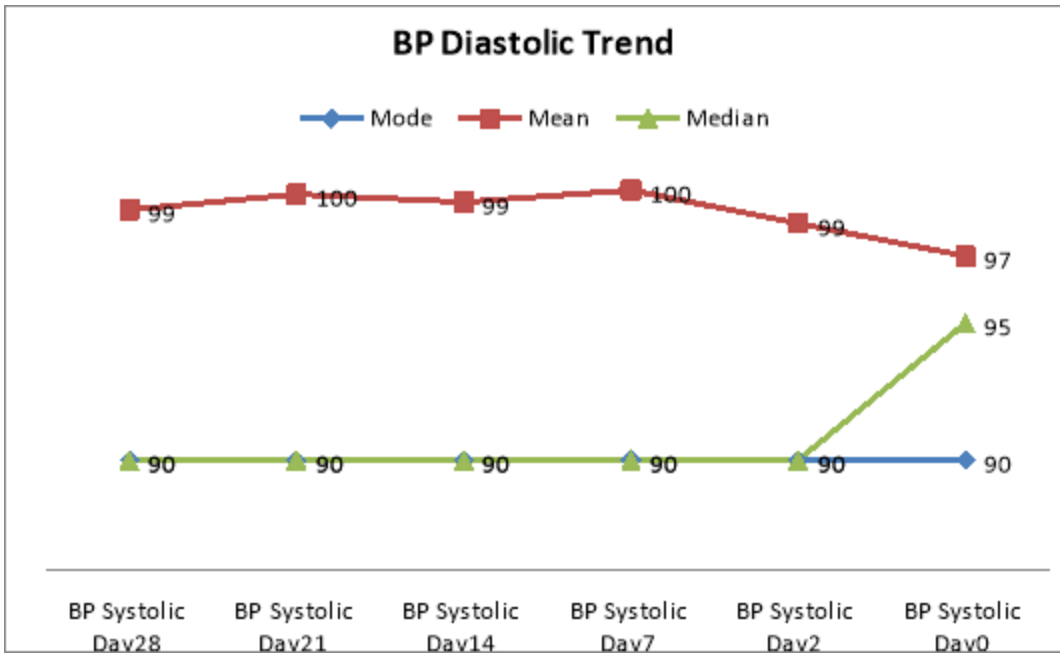
None of the above patients were received Primaquine and Chloroquine and the basophile was also zero

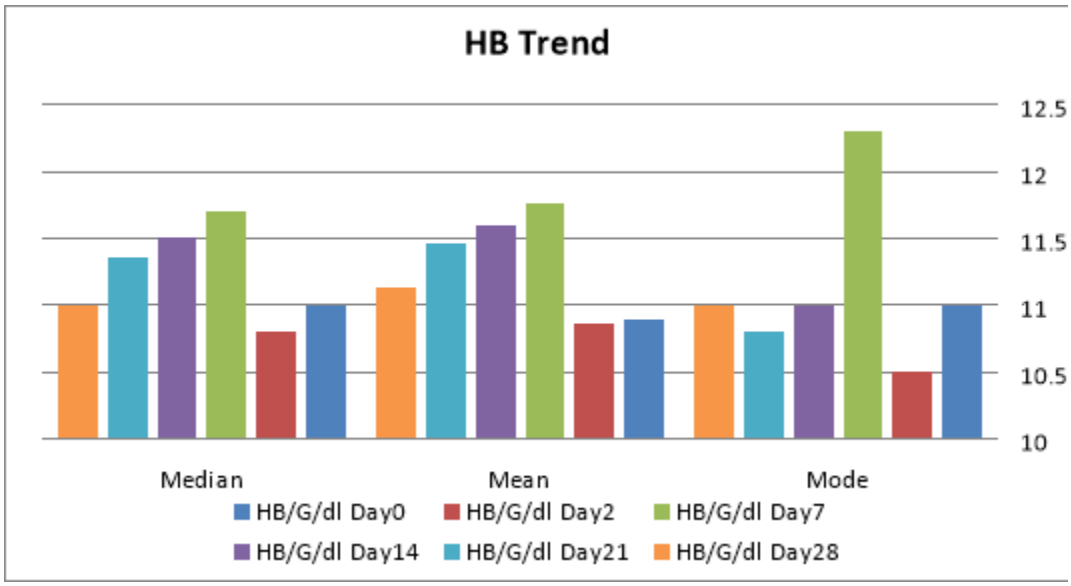
The result of day 28th is as follow:

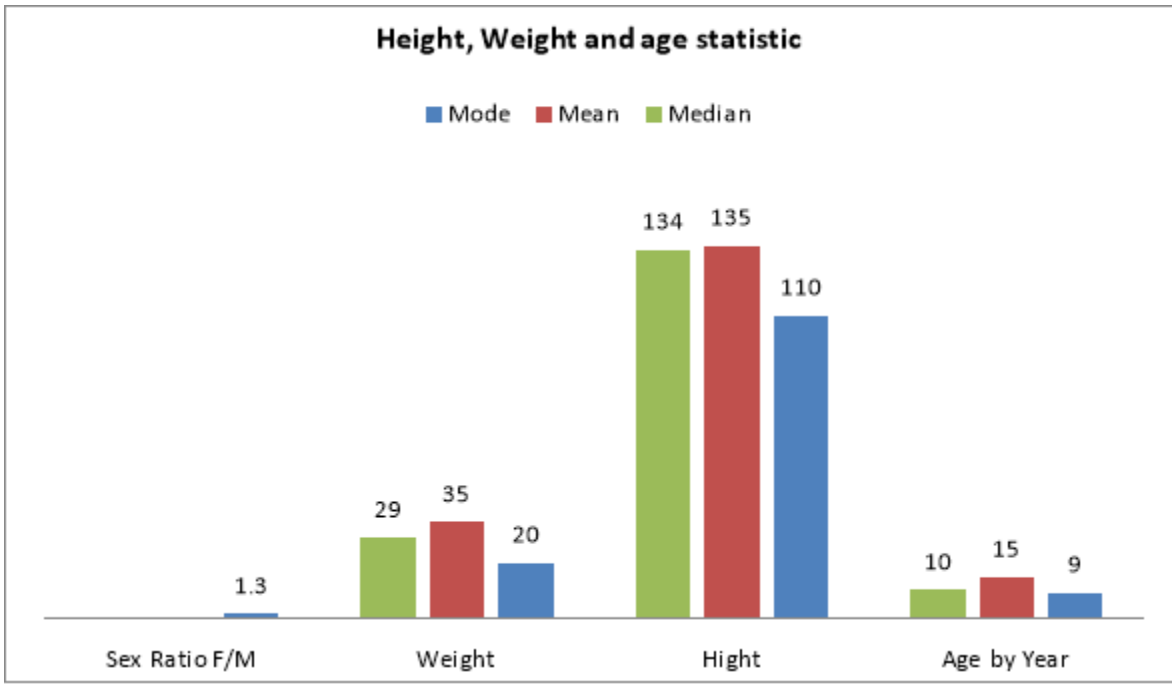
Statistic/indicators	Vital signs					Parasitemia		HB result	TLC	DLC					Medication	
	Temperature	RR	PR	BP Systolic	BP Diastolic	Trophosoitr	Gametocytes	gm/dl	Number	N %	L%	B%	M%	E%	CQ	PQ
Mode	36.5	16	75	90	70	0	0	12.3	7200	61	33	0	4	1	0	0
Mean	37	17	76	100	72	0	0	12	7097	61	34	0	4	1	0	0
Median	36.5	16	75	90	70	0	0	11.7	7200	61	33	0	4	1	0	0



The above chart shows temperature trend in different days of the study.


BP Diastolic Trend
Mode
Mean
Median
99
100
99
100
99
97
95
90
90
90
90
90
90
BP Systolic Day28
BP Systolic Day21
BP Systolic Day14
BP Systolic Day7
BP Systolic Day2
BP Systolic Day0


HB Trend
12.5
12
11.5
11
10.5
10
Median
Mean
Mode
HB/G/dl Day0
HB/G/dl Day2
HB/G/dl Day7
HB/G/dl Day14
HB/G/dl Day21
HB/G/dl Day28



Ethical Considerations:

Informed consent form:

Once a patient is identified by the malaria laboratory technician, he will inform the study doctor of the diagnosis. The patient (or parent) will then be administered with the informed consent form. There is an information section which explains the background to the study and what will happen to the patient. The form makes clear that participation is voluntary and that information is confidential. The patient signed or mark finger print the consent form and be given a copy.

Institutional ethical clearance:

- Do you have an ethical review board in your institution? Yes [√] No [ ]
- Institutional ethical clearance has been obtained for the study: Yes [√] No [ ]

Research was proposal was submitted to IRB and approved by IRB, therefore no conflict of interest is expected.

Required products:

Research reports: Reports will be shared with NMLCP and Maulana Azad University for MPH program

Mechanisms to ensure implementation of research results in the health policy of the concerned control programme of the Ministry of Public Health:

There are presently many efforts to improve malaria control and the National Treatment Guidelines are in the process of being upgraded. The data from this study will assist in formulation of the new guidelines. Secondarily, it is envisaged that this system would be used by providers of the general health services in Afghanistan (BPHS). If this intervention proves effective, it will be presented to the BPHS and health policy committees (Consultative Group on Health and Nutrition and the Technical Advisory Group) for consideration in the new round of BPHS policy.

Strategies to enhance the dissemination and utilization of results:

Data will be published in a peer reviewed, Malaria medical journal.

Timeline:

Task	Year 2018								
	Jan	Feb	Mar	Apr	May	Jun	July	Sep	Aug
IRB Approval									
Recruitment and training of staff									

Procurement of equipment and supplies									
Recruitment of patients									
Follow-up of patients									
Monitoring									
Data collection from clinics									
Completion of data entry									
Data analysis									
Report writing									
Submission of peer review article									
Final report									

BUDGET:

Budget Breakdown	Unit cost	Budget	Total (AFN)
Physicians Laboratory technician (incentive)	0	0	0
CHW Incentives	200	4000	4000
Monitoring	300	3000	3000
Water Bath (digital) x 2	5000	10000	10000
G6PD Tests (240)	0	0	0
HemoCue (1)	0	0	0
HemoCue Micro cuvettes (5 packs of 50)	0	0	0
Pipettes (2)	0	0	0
Filter tips (2 bags of 500)	0	0	0
Plastic tubes (50ml) (6 bags of 100)	0	0	0
Primaquine (15mg tabs, 200 adult doses)	0	0	0
Motorbike rental (2 motorbikes)	5000	10000	10000
Printing of forms	10	2500	2500
Perdiem Payments for Follow-up	300	6000	6000
GRAND TOTAL			**35500 AFN**

O. ***Consent Form:***

Title of Study: Operational assessment of referral for testing for glucose-6-phosphate dehydrogenase deficiency (G6PD) for administration of anti-relapse therapy for radical cure of vivax malaria.
Principle Investigator: Dr. Abdul Wakil Ziar BPHS Project Manager.
Tel No: +93799425181

Information and Consent:

You have been diagnosed with malaria caused by a type of malaria called vivax malaria. This disease has two forms, one which causes the symptoms you have now and one which nests in your liver and awakens to cause other episodes of malaria at some time in the future. Therefore, you may experience up to 6 or seven other episodes of disease, even if you are not bitten by any mosquitoes which transmit the disease – it may be nesting in your liver.

There is a treatment for the second stage of disease which will kill the nesting parasites. This treatment is called primaquine. If it is taken in a course for 14 days, it will cure the liver stage of disease and prevent you from getting other episodes. However, there is a problem with this treatment, which can cause side effects in people with a blood condition called G6PD deficiency. If you have this condition (which is in about 5-10% of the population), you could experience a bad reaction to the treatment. There is a test for this condition, which is available to you in the nearby clinic. If you take this test and you do not have the condition (G6PD deficiency) then you may take the medicine which will cure the liver stage of the parasite. If you do have the condition, you will be advised on what you should do by the study doctor at the laboratory.

If you agree to participate, we will strongly recommend that you go to the clinic and present the clinic head with a referral form that you will be given. They will perform as G6PD test and if you do not have the deficiency, you will be given primaquine for a 14-day course. The clinic will give you advice on how to take the medicine and some materials which will help you to remember to take the medicine. After two weeks you will be visited in your home

by a community health worker. He or she will ask you some questions, including whether you have had malaria or not. This will be repeated every month, once per month, for six months. At each visit we will take a blood-slide to confirm that you do not have malaria. This may cause mild discomfort, but will be done by a qualified person. We will ask you information about your history of malaria and other information that is used by us to help. You are also asked to come back to this clinic if you get sick with similar symptoms during the study period.

The aim of the study is to measure the number of episodes that are prevented by providing the G6PD testing service. If it is successful, the Ministry of Public Health will try to make the system available to all Afghans in areas where malaria is a problem. The benefit for you is that you will receive the free test and free treatment which prevent you from getting malaria from the liver stage of the vivax parasite You will also get medical advice and assistance during the follow-up period. The risk to you is mild discomfort from the medicine, which has been known to cause stomach upsets and some blood disorders. If you experience any severe symptoms you should telephone the duty doctor, Dr. who will make arrangements for assistance and treatment, including (if necessary), transfer to and treatment in hospital in Afghanistan.

Your participation is entirely voluntary and you may withdraw from the study at any time and for any (or no) reason. If you do not want to be part of the research you will be treated in the same way as normally in the clinic and it will not affect your access to healthcare now or in the future. All information that we collect will be completely confidential and will only be reviewed by those directly involved in the study. Your samples will be examined only for this study and will not be passed to any other person.

Do you understand what you have been told? Yes [ ] No [ ]
Do you have any other questions?
Do you agree to participate in the study: Yes [ ] No [ ]

Patient Signature or Mark:	**Doctor Signature:**	**Enroling Officer Signature:**
Date:	Date:	Date:

Economic and Socio-economic data:

Economic Data: Cost of:				
Transport	**Drugs**	**Diagnosis**	**Other treatments**	**Est. Loss of earning**

Socio-economic data:				
HH Assets:	TV [ ]	Radio [ ]	Car [ ]	Pressure Cooker [ ]
(tick all that apply)	Land [ ]	House [ ]	Business [ ]	
Education level:				
Number of rooms	[ ] [ ]	Number of people in HH	[ ] [ ]	
Education:	Illiterate [ ]	Literate [ ]	Secondary [ ]	Higher [ ]
Job of HH primary wage earner:	Unemployed [ ]	Unskilled [ ]	Skilled [ ]	Clerical / Professional [ ]

Adverse events profile:

Symptom	No / Yes	Symptom	No / Yes
Headache	No ○ Yes ○	Cyanosis	No ○ Yes ○
Vomit	No ○ Yes ○	Anaemia	No ○ Yes ○
Abdominal Pain	No ○ Yes ○	Dark coloured urine	No ○ Yes ○
Nausea	No ○ Yes ○	Convulsion	No ○ Yes ○
Diarrhoea	No ○ Yes ○	Skin Disorder	No ○ Yes ○

Monthly Follow-up:

Month	Malaria in reporting period	History of fever	Date of malaria or fever	Blood Slide Taken?	Slide result?	Patient has retained G6PD card?
1	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**
2	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**
3	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**
4	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**
5	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**
6	**Y / N**	**Y / N**	**/ /**	**Y / N**	**Positive / Negative**	**Y / N**

PAPER

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