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The Effects of Storage of *Falciparum* Infected Blood on Parasite Count, Haemoglobin and Bilirubin Level

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Abstract:
Objectives: *The main objective of this study is to study the effect of storage of falciparum infected blood on parasite count, haemoglobin and bilirubin level.*
Method: *This is Descriptive prospective study carried out in the central laboratory of Wad Medani Teaching Hospital .Sudan , All samples (100) were infected by P. falciparum & the parasite stages detected were rings form. For each sample, parasite count, haemoglobin estimation & bilirubin measurement were done immediately, after 7 days, 14 days & 21 days.The statistical analysis was done by using SPSS program.*
Results: Parasite count *: Parasite count in the immediate examination ranged between 150- 40000 parasite/µl of blood, the mean was 1558.5.Then it started to decreased after 7 and 14 days to become no parasite after 21 days.*
Haemoglobin level *: The mean of haemoglobin in immediate estimation was 80% & 70% for males & females respectively. The samples showed decreased in haemoglobin level in both sex after 7, 14 and 21 days*
Bilirubin estimation*: in immediate estimation From the 100 samples 42 samples showed increased in bilirubin level. After 7 days 78 samples showed normal bilirubin level, & 22 samples showed mild decreased due to exposure to sun light, but still in abnormal level. After 14 days most samples showed normal bilirubin level ,only 5 samples remain in the increased level. On day 21 all samples were in the normal level.*
Conclusion: *the study concluded that storage of infected blood by P.falciprum leads to hemoglobin fall down due to utilization by the parasite.Storage of infected blood by P.falciprum leads to decease in parasite count & after 21 days the blood becomes negative.Storage of infected blood by P.falciprum leads to bilirubin fall down due to exposure to sun light which causes destruction of bilirubin.*

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I. Introduction

Malaria is a vectro – borne illness caused by intracellular protozoan parasite belong to the genus plasmodium,it is a major public health problem in tropical areas which lead to a potentially fatal acute febrile illness following invasion and multiplication in human red blood cells (RBCs) during their complex life cycle. In many endemic areas it is becoming too difficult to control because of the resistance of the parasite to anti malarial drugs and the failure of vector control measures. [(1)] .Five species of *Plasmodium* are currently known to cause malaria in humans: the deadliest *Plasmodium falciparum* and *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi*. Malaria parasites transmitted through the infective bites of female *Anopheles* mosquitoes when it takes blood meal. Transmission of malaria via blood transfusion is the second route, in which the sexual cycle & tissue phase are omitted [(2)]. It was first reported in 1911 by Woolsey.[(3)] It occur by the transfusion of whole blood or a blood component from a malaria infected donor to a recipient . Also transmission via donated kidneys & erythrocytes concentrates has been reported .[(4)]. [(*Plasmodium falciparum*, *P. vivax* and *P. malariae* are the most frequently species detected in TTM [(5)]. According to Kinde-Gazard *et al*, 33.5% of donors carried various species of malaria parasites, but had no symptoms [(.6)]. Malaria parasites of all species can remains viable in stored blood for at least one week [(7)].A case of *P. falciparum* malaria has been transmitted by stored blood for 14 days [(8)], in another case *P .falciparum* remains viable in stored blood for 19 days [(9)]. Also the parasites may even survive well in frozen blood [(8)]. According to Miller (1975) *P .falciparum* infections are usually eliminated within one year by the host immune mechanism. *P. falciparum* transmitted from an asymptomatic donor can cause death in a non-immune recipient. However, transfusion malaria in non endemic areas is an uncommon complication of blood transfusion, but because of delay in diagnosis, transfusion malaria has a relatively high fatality rate. It can be particularly serious in pregnant women, splenectomized and immunocompromised patients [(10)].

II. Methodology

Definition of the study:

This is a descriptive prospective study, to find out the effect of storage of *falciparum* infected blood on falciparum malaria parasite count, haemoglobin and bilirubin level. The study was conducted during the period from July 2005 to July2007.

Study area

The study was carried out in the central laboratory of Wad Medani Teaching Hospital. Rebublic of Sudan Wad Medani city locate in the Blue Nile and its an agriculture and migrated area, where Immigrants came from other malaria endemic area.Also this city reported high incidences of malaria cases especially in Winter season.

Study Design

This was a descriptive study. Blood samples were collected in sterile containers containing Citrate phosphate dextrose adenine (CPDA) ((the common used anticoagulant in Sudanese blood bank) & examined for malaria parasites by thick & thin blood films, parasite count, measurement of haemoglobin & bilirubin level were done for each samples immediately, after 7 days, 14 days & 21days.

The criteria for selection of study cases

Sample size

100 *falciparum* blood samples (but actually dealing with 400 tests, because each sample is tested four times).50 blood samples were collected from volunteers & used as controls.

Inclusion Criteria

Adult with age raging between 20-40 years. (the common donation age).

Positive *falciparum* blood samples, with any degree of parasitaemia.

Exclusion Criteria

Haemolysed samples.

Steps of study

For each blood sample the following was done:

Thick & thin blood films were prepared, then stained by Geimsa stain (with concentration of 10% & the staining time was 10 minutes), the stained films were assessed by a technologist experienced in malaria microscopy.

Haemoglobin was measured by using colorimetric method (each sample done three times then mean was taken to avoid technical errors & haemoglobin standard was performed).

Bilirubin measured using kits method according to the manufacture instructions.

To detect the effect of storage of falciparum infected blood on parasite ,, the mentioned tests above were done immediately, after 7 days, 14 days & 21days.

Methods

Collection of specimens

From each patient & voleenters, 3 ml of venous blood was collected in sterile containers containing 0.42 ml CPDA anticoagulant solution which consisted of sodium citrate, citric acid, sodium phosphate, dextrose, adenine & water. After immediate preparation of the above tests, the samples were preserved in refrigerator at 4^{o}C to keep them in condition similar to that of the blood bank.

Thick & thin blood films prepration

Thick & thin blood films were prepared by the standard method according to Monica Gheesbrough immediately, then after 7 days, 14 days & 21 days. Then were stained by 10% Geimsa stain for 10 minutes**,** and then examined by a microscope using oil lens [(1)]. Estimating parasite numbers/µl of blood was done by counting parasites against WBCs as follows:

From the thick blood film, well stained parasites & well distributed WBCs part of the film was selected, by using the oil immersion objective the parasites were counted against 200WBCs, then using the following formula to estimate the parasite count :

$$\frac{\text{Numbers of parasite counted X Total white blood cell}}{200}$$

Haemoglobin measurement

Measurement of haemoglobin was done immediately, after 7 days, 14 days & 21 days by colorimetric method (cyanmethhaemoglobin method according to S.M.Lewis).

Principle of the test

The base of the test is dilution of blood (0.02 ml) in drabkin solution (4 ml) which is containing of 200 mg potassium cyanide & 50 mg potassium ferricynide & litre of water.Haemoglobin ,HiCN & HbCOconverted to HiCN ,the absorbance of the solution measured by a colorimeter using a filter of 520 nm.For each sample haemoglobin was measured three times then mean was taken to avoid technical errors & haemoglobin standard was used [(11)].

Bilirubin measurement

Total bilirubin was measured immediately, after 7 days, 14 days & 21 days.

Principle of the test

Bilirubin converted to coloured azobilirubin by diazotized sulfanilic acid .

Procedure

For each sample four tubes were prepared as mentioned in the following table as instructed by the manufactures in the kits (the kits were obtain from linear chemicals –Spain)

Tubes	Reagent blank	Sample blank	Sample	CAL
Distilled water	100 µl	_	_	_
Sample	_	100 µl	100 µl	_
CAL*	_	_	_	100 µl
R T**	_	1 ml	_	_
Working reagent***	1 ml	_	1 ml	_

* CAL means bilirubin calibator which was dissolved in 1 ml of distilled water, mixed & let stand for 5-10 mintes before use.

** RT mean reagent, it is sulfanilic acid, which converts bilirubin tocoloured azobilirubin

*** Working reagent prepared by mixing of 1 ml of sodium nitrite & 4 ml of sulfanilic acid.

The tubes were mixed & let stand for 2 minutes at room temperature, then the absorbance of each tube was read by colorimeter using 540 nm filter.

Calculations:

Absorbance of sample-- Absorbance of sample blank X Absorbance of CAL

Statistical analysis

The statistical analysis was done by using SPSS program.

Ethical consideration

The ethical clearance was obtained from the ethical committee, ministry of health. Gazira state. The patient also gave verbal informed consent

III. Results

1Distrbution of age & sex

100 blood samples were collected from malaria patients, 59 males aged 20-40 years & 41 females aged 20-38 years as shown in (table 1 & table 2).

Table (1) shows sex distribution in infected samples:

Total numbers of samples	100
Males samples	59
Females samples	41

Table (2) shows the age distribution:

Males	20-40 years
Females	20-38 years

Result of laboratory tests

All samples were infected by *P. falciparum* & the parasite stages detected were rings. For each sample, parasite count, haemoglobin estimation & bilirubin measurement were done immediately, after 7 days, 14 days & 21 days.

Parasite count

Immediately after collection

Parasite count in the immediate examination ranged between 150- 40000 parasite/µl of blood, the mean was 1558.5 (table 3).

After 7 days

As indicated in (table 3) the parasite count fell down, the high count of parasitemia was 9900 & the lowest one was 100 parasites /µl of blood, the mean was 562.5.

After 14 days

There was highly decreased in parasite count, the highest count of parasitaemia was 1250 & the lowest one was 40 parasite /µl of blood, the mean was 191.4 (table 3).

On day 21

On day 21 no parasites were detected (table 3).

Table (3) shows the mean of parasitaemia/µ of blood for both sexes:

Immediate examination	1558.5
After 7 days	562 .5
After 14 days	191 .4
After 21 days	0

Haemoglobin estimation

Immediately after collection

The mean of haemoglobin in immediate estimation was 80% & 70% for males & females respectively (table 8 & 9).

After 7 days

The samples showed decreased in haemoglobin level in both sex, 10 males & 14 females samples became low, the mean was 72% & 65% respectively (table 8 & 9).

After 14 days

Most of females samples (33 samples) & 25 males samples showed low haemoglobin level , the mean was 64% & 57% (table 8 & 9).

On day 21

All females' samples & 40 males' samples showed low haemoglobin level & as mentioned in (table 8 & 9) the mean was 57% & 51%.

Table (7) shows the mean of haemoglobin level in males (g/dl & %)

Immediate measurement	11.8 (80%)
After 7 days	10.5 (72%)
After 14 days	9.3 (64%)
After day 21	8.6 (57%)

- **P value > 0.05**

Table (8) shows the mean of haemoglobin level in females (g/dl & %):

Immediate measurement	10.4 (70%)
After 7 days	9.7 (65%)
After 14 days	8.6 (57%)
At day 21	7.5 (51%)

P value > o.o5

Bilirubin estimation

The normal bilirubin level for adult is 1.1 mg/dl.

Immediately after collection

From the 100 samples 42 samples showed increased in bilirubin level (fig. 8).

After 7 days

As mentioned in (fig. 9), 78 samples showed normal bilirubin level, & 22 samples showed mild decreased due to exposure to sun light, but still in abnormal level.

After 14 days

Most samples showed normal bilirubin level as mentioned in (fig. 10) & only 5 samples remain in the increased level.

On day 21

All samples were in the normal level.

Table (9) shows the mean of bilirubin level (mg/dl):

Immediate estimation	2.34
After 7 days	1.94
After 14 days	.62
After 21 days	.32

Results of control samples

50 blood samples negative for malaria parasites were used as controls (30 males & 20 females)**.**

Parasite count

All samples (50 samples) were negative for malaria parasites.

Haemoglobin estimation

The base line level of haemoglobin in the immediate estimation was 15.4 g/dl for males & 12.6 g/dl for females, there was no change in haemoglobin level during storage.

Bilirubin measurement

In the immediate estimation all samples were in normal range (up to 1.1 mg/dl), after
Storage they showed decreased due to exposure to sun light as mentioned in (table 10)

Table (10) shows mean of bilirubin level in control samples (mg/dl)

Immediate estimation	1.05
After 7 days	0.74
After 14 days	0.54
On day 21	0.32

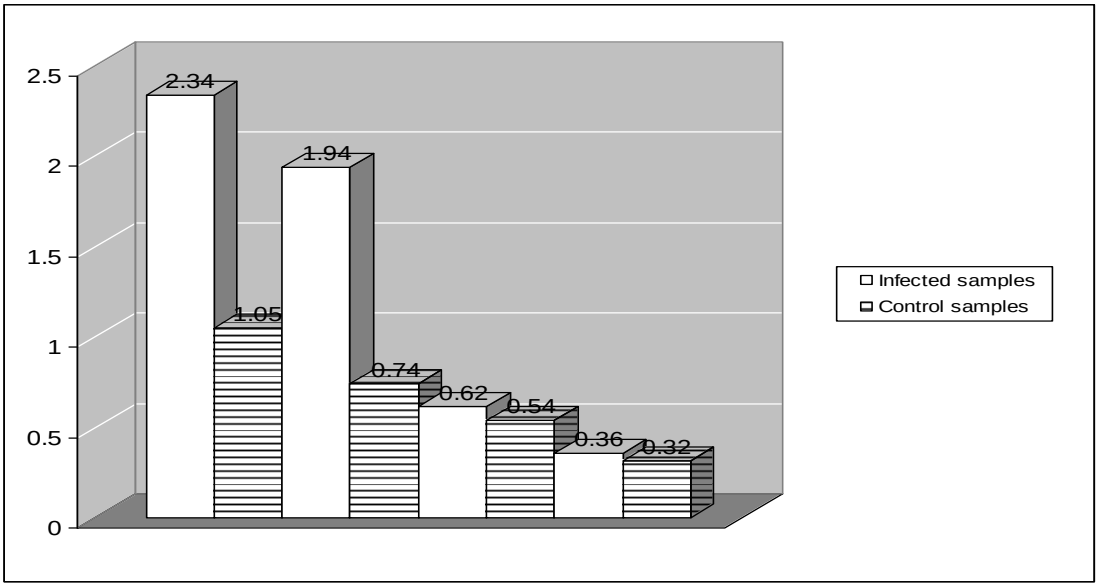

Figure (11) shows comparison of bilirubin level between control & infected samples

- value > 0.05 (after 7 &14 days)
- No significant difference after 21 days.

IV. Disscussion:

Several types of infectious organisms can be transmitted through blood transfusion. Cases of imported malaria are increasing world wide due to modern traveling & increased demand of blood transfusion. Transfusion-transmitted malaria compared to natural infection often has a short incubation period because there is no pre-erythrocytic development .Several reports showed that all *plasmodium* spps can remain viable in stored blood for at least one week, whereas *P.falciprum* parasite has been transmitted by blood stored for 19 days , but with less potential for infection, and therefore older blood can still transmit malaria, but to a lesser extent [2-8-12-13]

In this study identification of malaria parasite was done by using the standard microscopy technique [2] & although some studies showed low sensitivity, but it is cheaper than using other sensitive methods like molecular methods.

As shown in (table3), the samples showed slight to moderate parasite count on the immediate examination (150-40000 parasite /µl of blood), it was easy to find malaria cases as Gazira is an endemic area of malaria & especially samples were collected in the rainy season. Such infected blood transferred to a patient , it may lead to serious complications & may lead to a potentially fatal outcome ,because screening policy of examination of blood for malaria parasite is not included in Sudanese blood bank . Transfusion malaria is serious because the recipient most of the time are patient weakened by severe other illness ([14, 15].

The parasite count started to drop gradually & on day 21 the parasite disappeared from the stored blood & the blood became negative for malaria parasite. It is difficult to find a simple explanation to this. One possibility is the even dropping level of haemoglobin .Another is the in vitro environment with lack of replenishment of nutrients & accumulation of toxic metabolic products & possibly limited O_2 & glucose supplies.

De Silva M & Contreras M, Barbara (1988) showed that *P. falciparum* malaria has been transmitted by blood stored for 19 days. ([9]

Woolsey in 1911 reported that malaria parasites could be transmitted by the transfusion of any blood component containing red blood cells & parasites of all species could remain viable in stored blood for at least one week [2].

In a study in 2004 done in the blood bank of Wad Medani Teaching Hospital ,Central Sudan (2004) using conventional method (microscopic examination & RDTs) & the molecular method (QT-NASBA), demonstrated that microscopy & RDTs are not sensitive enough to detect low parasitaemia in apparently healthy donors .[16]

Ali M S, etal in 2005 showed that screening donors for malaria prior to donation will undoubtedly reduce the risk of malaria infection & microscopy was much cheaper than both ICT and PCR; in addition, it was possible to detect all human *plasmodium* species [(15)].

In another study done in Ahmed Gasim hospital in Khartoum capital Sudan in 2001, among blood donors screened for malaria parasites by thick & thin blood films using Geimsa staining technique, the incidence among donors was 6.5%, the majority of them were between 20-40 years old, the peak age for donation & it is the same age range selected in this study , *P. falciparum* contributed to 98% & the 2% were *P .vivax* [(14)].

The shelf life of blood is 35 days & haemoglobin usually remains unchanged for days, provided that blood is not infected .As yet no study had been carried out to determine the effect of storage of infected blood on haemoglobin measurement. In the present study, the male samples, in the immediate estimation of haemoglobin showed a higher level than that of females samples , after 7 days the haemoglobin started to decrease , this is most likely due to its utilization by the parasite which is known to feed on haemoglobin .After 14 days there was marked decrease in haemoglobin level & most samples became a quite low , especially females samples. This may be due to the fact that the mean of parasitaemia in females samples was higher than that of males sample. On day 21,all females samples had low mean of haemoglobin & only nineteen males samples were in the base line level .The control samples (50 samples) showed stable haemoglobin level during storage period [(11)] , & there was significant difference between controls & infected samples .

The study also tried to find out the changes of bilirubin level during blood storage which to our knowledge is not done before. In the immediate estimation ,58 infected samples showed high bilirubin level ,this was due to destruction of RBCs by the parasite which lead to hyperbilirubinaemia [(1)], then bilirubin level decreased weekly ,this may be due to storage & exposure to sun light ,which caused destruction of bilirubin [(1)].The control samples showed normal level at the immediate estimation ,then started to decrease weekly .There was significant difference between control & infected samples at the beginning , this may be due to the fact that all control samples were in the normal range, whereas some of the infected samples showed increased in bilirubin level .On day 21 there was no significant difference between control & infected samples .The decreased of bilirubin during the storage period may also due to there is no bilirubin synthesis in vitro as there is no liver role .

In study done in1992 in Taipei, to find out the effect of light on total bilirubin in vitro, concluded that blood samples for bilirubin should not exposure to light, & if there was delayed in measurement the samples should be saved in dark environment [(17)].

V.Conclusion

- Microscopic examination is the cheaper, sensitive technique to be used in Sudanese blood bank.
- Storage of infected blood by *P.falciprum* leads to hemoglobin fall down due to utilization by the parasite.
- Storage of infected blood by *P.falciprum* leads to decease in parasite count & after 21 days the blood becomes negative.
- Storage of infected blood by *P.falciprum* leads to bilirubin fall down due to exposure to sun light which causes destruction of bilirubin.
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Vi. RECOMMENDATIONS

- Screening of blood donors for malaria parasite should be included in the protocol of Sudanese blood bank.
- Transfusion-transmitted malaria should be considered in transfusion recipients who have undiagnosed symptoms consistent with malaria, such as fever or chills, and thick and thin blood smears should be urgently examined to exclude this possibility.
- Further studies should be done to find out the superior, accurate & cheap method for screening donor's blood for malaria parasites before transfusion.
- These recommendations should be circulated to Sudanese blood banks.

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