Omdurman Islamic University Journal(OIUJ)
مجلة جامعة أم درمان الاسلامية
https://journal.oiu.edu.sd/index.php/oiuj
https://doi.org/10.52981/oiuj.v17i2.1844
2019; 17(2):198-210
ISSN: 5361-1858

The Predictive Values of Lymphocytopenia in West Darfur Patients with Malaria

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To cite this article:

Salma Mohammed Abotaha1, Amira Ahmed Khalid Humeida (MBBs- MD). The Predictive Values of Lymphocytopenia in West Darfur Patients with Malaria , Omdurman Islamic University Journal,Vol. 17, No. 2, 2021, pp. **198-210,** https://doi.org/10.52981/oiuj.v17i2.1844

Received*:05,11, 2021;* ***Accepted****:10,20, 2021; Published: 12,27, 2021.*

ABSTRACT

Background: In Sudan malaria is most commonly caused by infection with plasmodium falciparum, although by p.vivax. Malaria causes the most dangerous and highest rates of complication and mortality. Most malaria cases in 2018 reported by world health organization (WHO) in African region(213 million cases of malaria or 93% from all malaria cases in world and 70% being 5 years or younger).

Objectives: The aim of this study was to measure and compare the mean of absolute lymphocyte count in malaria patients and control group, and to determine positive and negative predictive values of lymphocytopenia in malaria patients.

Methods: It was conducted on 100 subjects with malaria as cases and 100 subjects without malaria as controls, at EL Genina Hospital after obtaining the ethical approval and the subjects' consent. It was done by testing the CBC, differential counts and absolute lymphocyte count then determining the means and p-values. The positive and negative predictive values were also determined.

Results: It was found that the mean of TWBC count in case group was(7,13109/l) ,and (7,84109/l)in the control group ,p value was (0.150). The mean of lymphocytes differential in case group was (20.73%)and (33.96%)in the control group, p-value was (0.000). While the mean of the absolute lymphocytes count in case group was (1.39109/l),it was (2.56109/l)in the control group, with a p value (0.000). This p-value indicated that there was significant lymphocytopenia in malaria patients. The positive predictive value was 83% and negative predictive value was 69%.

Conclusion: This study concluded that there was nosignificantlymphocytopenia in malaria patients, and that lymphocytopenia cannot be used as the key haematological indicator of malaria infection.

Keywords: The Predictive Values, Lymphocytopenia, West Darfur, Malaria

1.Introduction:

Malaria constitutes a major public health problem in Sudan. Almost, 75% of population is at risk of developing malaria. In 2016 showed an overall parasite prevalence of 5.9%.in South Darfur, West Darfur, Blue Nile, and South Kordofan State the prevalence approached or exceeded 10%. Children are 3 times more likely to get malaria than adult. Apparently there was no difference between male and female. (Mosab,2017,23) Increase in malaria incidence rates among population in 2019 accounting for 12,4 percent of all diseases, with a mortality rate of 13per 10,000.(Sudan Situation Report, 2019, 6)

Malaria is caused by infection of red blood cells with protozoan parasites of the genus plasmodium inoculated in to human host by feeding female anopheles mosquito. Five plasmodium species transmitted from person to person(p.falciparum , p.vivax , p.ovale two species, p.malariae),increasingly human infection with the monkey malaria parasite p.knowlesi are being reported from South-East Asia and particularly the island of Borneo.(WHO, 2015,1211) Recent WHO report estimated 95% and 5% of p.falciparum and p.vivax malaria cases respectively in Sudan.(Makarim,2016,4)Malaria is suspected when patient present with fever(or history of fever within 48 hours)with or without other symptoms and signs suggestive of malaria(e.g headache, vomiting, sweating).(Global Malaria Program,2012)Patients were classified as having severe malaria if they met one or more of the WHO criteria for severe malaria, either on presentation or later during hospital admission.(World Malaria Report,2019,31)Severe malaria is most commonly caused by infection with plasmodium falciparum, although by p.vivax and p. Knowles. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction.(Marilues E,2013,12)

The white blood cell may be divided in to two broad : the phagocytes comprise the cell of the innate immune system, which can act very quickly after an infection , and lymphocytes mediate the adaptive immune response, which can develop immunological memory. Phagocytes themselves subdivided into granulocyte (which include neutrophils, eosinophil, basophils) and monocytes. The functions of phagocytes and lymphocytes in protecting the body against infection.(WHO,2014,131)Lymphocytes are the immunological competent cells that assist phagocytes and defence of body against infection and other invasion.(Srisurapanon S, 2013,34) Two unique features characteristic of the immune system are the ability to generate antigenic specificity and phenomenon of immunological memory .The bone marrow and thymus are primary lymphoid organs in which lymphocytes develop, the secondary lymphoid organs in which specific immune response are generated are the lymph nodes, spleen and lymphoid tissues of the alimentary and respiratory tracts.(MARK J,1995,316)

Two types of predictive value exist, the positive predictive value or precision rate is defined as a proportion of people with a positive test who are actually ill.(Victor hoffbrand,2016)The positive predictive value of the test is high if the disease prevalence is high, and low if disease prevalence is low. (Stojanovic, 2014,11) and negative predictive value is defined as a proportion of people with a negative test result who actually do not have disease.(John Wiley,2014,131)

Objectives:

To measure and compare the mean of absolute lymphocyte count (ALC) in malaria patients and control group, to determine positive and negative predictive value of lymphocytopenia in malaria patients, to correlate lymphocytopenia between the age and gender, and to correlate lymphocytopenia between different level of parasitemia(+,++,+++,++++).

Materials and Methods:

Analytic - Case control study was carried out in El Genina Hospital in West Darfur state, was selected using instructed questionnaire including variable as (age , gender).During study period 100 subjects with malaria as cases and 100 subjects without malaria as control were tested for lymphocytopenia. The study included patients with malaria, diagnosed by microscope who were new cases not on treatment, the age of patients ranged from 18to 40 years. Any patients without malaria and any patient refused to share with us was excluded.

Three ml venous blood was collected from each patient and control group by standard procedure in EDTA container. The study included200 patients, 100 subjects with malaria and 100 subjects without malaria as control.Complete blood cell count (CBC) was done. The value of white blood cell (TWBC), and lymphocyte percentage (lymphocyte %) was taken. The ALC will be expressed in thousands of cell per micro liter, by the equation: absolute lymphocyte count (ALC) =WBC*lymphocyte %.and the predictive value (positive or negative predictive value) was estimated by equation: predictive value of positive = (TP/TP+FP) X 100, and the predictive value of negative = (TN/FN+TN) X 100. TP is true positive disease present, FP is false positive disease absent, FN is false negative disease present, TN is true negative disease absent.

Results:

The TWBC count was measured in malaria patients and control group, which showed that 7% of all malaria patients and 5% from control group had leukocytopenia, while 9% of all malaria patients and 9% from control group had leukocytosis, as showed in table1.The mean of TWBC count in case group was(7,13X10^9/l),and (7,84X10^9/l)in the control group ,p value was (0.150)as showed in table2.

The lymphocytes differential was measured in malaria patients and control group, which showed that 55 % of all malaria patients and 7% from control group had lymphocytopenia, while5% of all

malaria patients and 24% from control group had lymphocytosis, as showed in table 3. The mean of lymphocytes % in case group was(20.73%),and (33.96%)in the control group ,p value is (0.000)as showed in table 2.

Absolute lymphocyte count (ALC) showed that 61 % of all malaria patients and 12% from control group have lymphocytopenia, as showed in table 4, while the mean of the absolute lymphocytes count was reported as ($1.39X10^9$/l) ,and ($2.56X10^9$/l)in the case group and control group respectively, with a p value of (0.000) as shown in table 2.

The TWBC count in (+) parasitemia patients was($6.79X10^9$/l),($6.43X10^9$/l) in (++) parasitemia,($7.20X10^9$/l) in (+++)parasitemia , and ($7.94X10^9$/l) in (++++) parasitemia, with a p-value0.634.The lymphocytes % in (+) parasitemia patients was(24.5%),(18.3%) in (++) parasitemia,(18.7%) in (+++) parasitemia , and (22.0%) in (++++) parasitemia, with a p-value0.015.The mean of absolute lymphocytes count in (+) parasitemia patients was($1.48X10^9$/l),($1.34X10^9$/l) in (++) parasitemia,($1.20X10^9$/l) in (+++) parasitemia , and ($1.58X10^9$/l) in (++++) parasitemia, with a p-value.442as showed in table 5.

The TWBC count in males was($7.52X10^9$/l) and ($6.77X10^9$/l) in females, the p value was 0.361. The lymphocytes % in males was(21.81%) and (19.92%) in females, the p value was 0.396.The mean absolute lymphocyte count in males was($1.38X10^9$/l) and ($1.39X10^9$/l)in females, the p value was 0.922 as showed in table 6 .

The mean of TWBC counts according to age of patients from (20-25),(25-30),(30-35),(35-40) were ($7.70X10^9$/l),($7.30X10^9$/l),($6.21X10^9$/l) and ,($6.84X10^9$/l) respectively, the p-value was 0.760 , lymphocyte percentages were (21.8%),(20.5%),(18.7%) and (17.9%) respectively with a p-value of 0.083. The mean of absolute lymphocytes counts were ($1.73X10^9$/l),($1.16X10^9$/l),($1.62X10^9$/l) and(1.64)respectively and the p-value was 0.843 as showed in table (7). When compared to control group, the TWBC counts according to age showed means of ($8.05X10^9$/l),($7.95X10^9$/l),($7.80X10^9$/l) and ($7.85X10^9$/l) respectively, p-value was 0.650 , lymphocyte% were (34.6%),(32.2%),(32.9%) and (30.8%), a p-value of 0.078. The absolute lymphocytes counts were ($2.58X10^9$/l),($2.62X10^9$/l),($2.53X10^9$/l) and (2.50)respectively ,p value was 0.905 as showed in table (8).The R value was-0,0032 ,the p value was 0.300.

In considering the total number of 100 malaria patients and 61 TP cases, there were 39 cases of FN, in addition , total number of negative test was equal 127, therefore number of TN cases =127-39=88. Based on the above-mentioned calculations screening performance characteristics of lymphocytopenia in malaria patients were as followed:

- Sensitivity:61/100=61%
- Specificity:88/100=88%

- Accuracy:61+88/200=74%
- PPV:61/73=83%
- NPV:88/127=69%

Table 1:Percentage of leucopenia and leucocytosis in case and control groups

TWBC	**Leucopenia %**	**Leucocytosis%**
Case	7	9
control	5	9

Table 2: Mean results of TWBC, lymphocyte% and absolute lymphocyte in case and control

Parameters	**Case (Mean±SD)**	**Control (Mean±SD)**	***P-value***
TWBC	7.13±4.09	7.84±2.96	0.150
Lymphocyte %	20.73±11.07	33.96±9.76	0.000
Absolute lymphocyte	1.38±0.91	2.56±1.12	0.000

Table 3:Percentage of lymphocytopenia and lymphocytosis in case and control

Lymphocyte %	**low lymphocyte%**	**high lymphocyte %**
Case	55	5
control	7	24

Table 4:Percentage of absolute lymphocyte count in case and control

Absolute Lymphocyte	**Lymphocytopenia**	**Lymphocytosis**
Case	61	3
Control	12	20

Table 5: Mean results of TWBC, lymphocyte and absolute lymphocyte in different parasitemia levels

	Mean±SD				***P-value***
	+	++	+++	++++	
TWBC	6.79±4.54	6.43±2.34	7.20±4.79	7.94±3.86	0.634
Lymphocyte %	24.5±12.4	18.3±10.8	18.7±9.71	22.0±10.9	0.015
Absolute lymphocyte	1.48±1.15	1.34±1.00	1.20±0.71	1.58±0.77	0.442

Table 6:Mean count of TWBC, lymphocyte % and absolute lymphocyte in relation to gender

Parameters	Male (Mean±SD)	Female (Mean±SD)	*P-value*
TWBC	7.52±5.05	6.77±3.02	0.361
Lymphocyte %	21.81±12.23	19.92±9.94	0.396
Absolute lymphocyte	1.38±0.76	1.39±1.02	0.922

Table7:Mean count of TWBC, lymphocyte% and absolute according to the age of malaria patients

	Case(Mean±SD)age				*P-value*
	20-25	**25-30**	**30-35**	**35-40**	
TWBC	7.70±3.44	7.30±3.43	6.21±4.55	6.84±4.66	0.760
Lymphocyte %	21.8±22.4	20.5±10.5	18.7±36.6	17.9±12.5	0.083
Absolute lymphocyte	1.73±1.45	1.16±1.55	1.62±0.71	1.64±0.67	0.843

Table 8:Mean count of TWBC, lymphocyte% and absolute lymphocyte count according to the age of control group

	control(Mean±SD)age				*P-value*
	20-25	**25-30**	**30-35**	**35-40**	
TWBC	8.05±4.33	7.95±4.34	7.80±5.54	7.85±6.44	0.650
Lymphocyte %	34.6±21.3	32.2±13.5	32.9±10.6	30.8±10.5	0.078
Absolute lymphocyte	2.58±1.03	2.62±1.34	2.53±1.07	2.50±0.76	0.905

Table 9:Mean age of case and control

Variable	Group	Mean±SD	*P-value*
Age	Case	29.82±6.83	0.596
	Control	29.32±6.49	

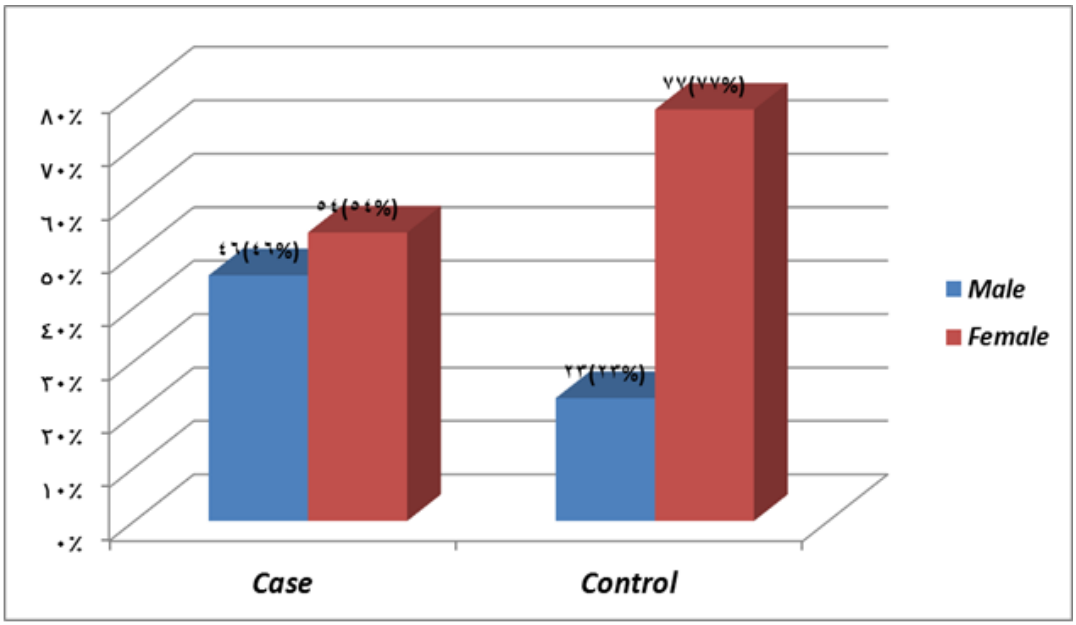

Figure 1: Comparison of gender between case and control groups

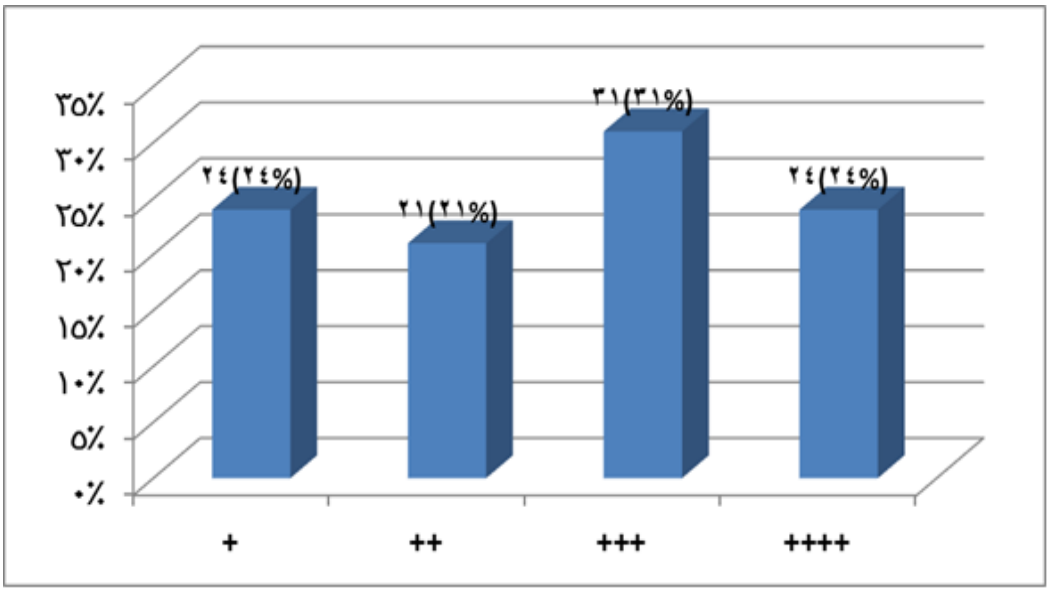

Figure 2: Parasitemia frequency in malaria patients

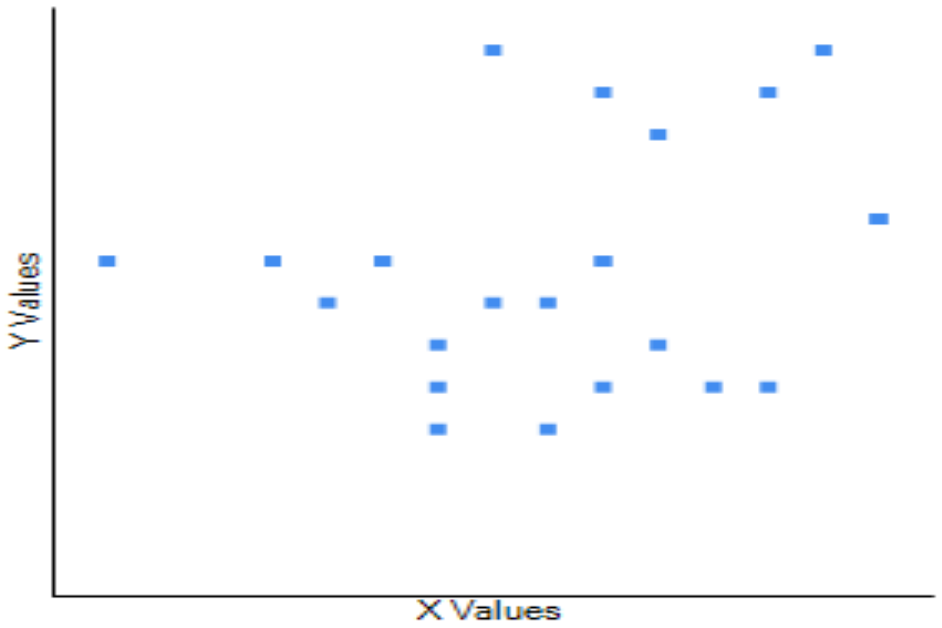

Figure3: Correlation between lymphocytopenia and parasitemia

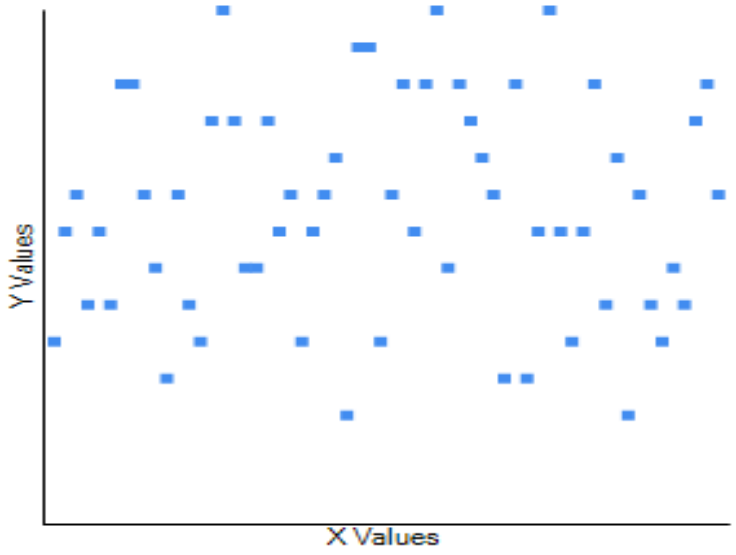

Figure 4: Correlation between lymphocytopenia and gender

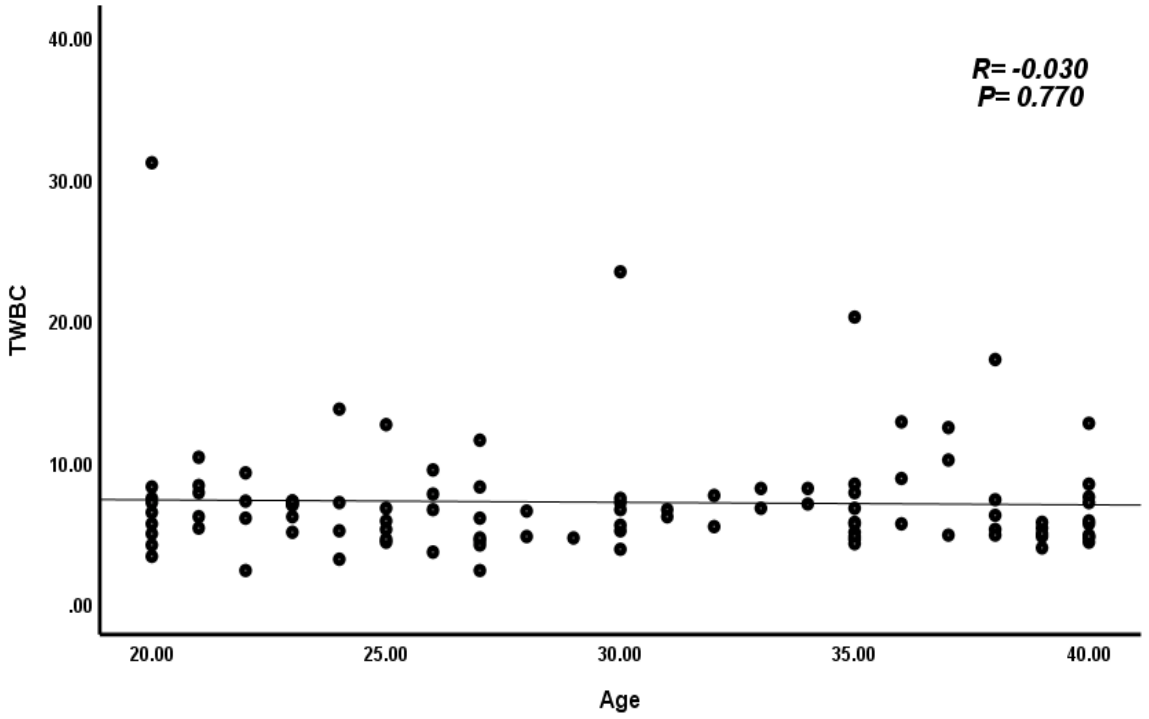

Figure 5: Correlation between TWBC count and age

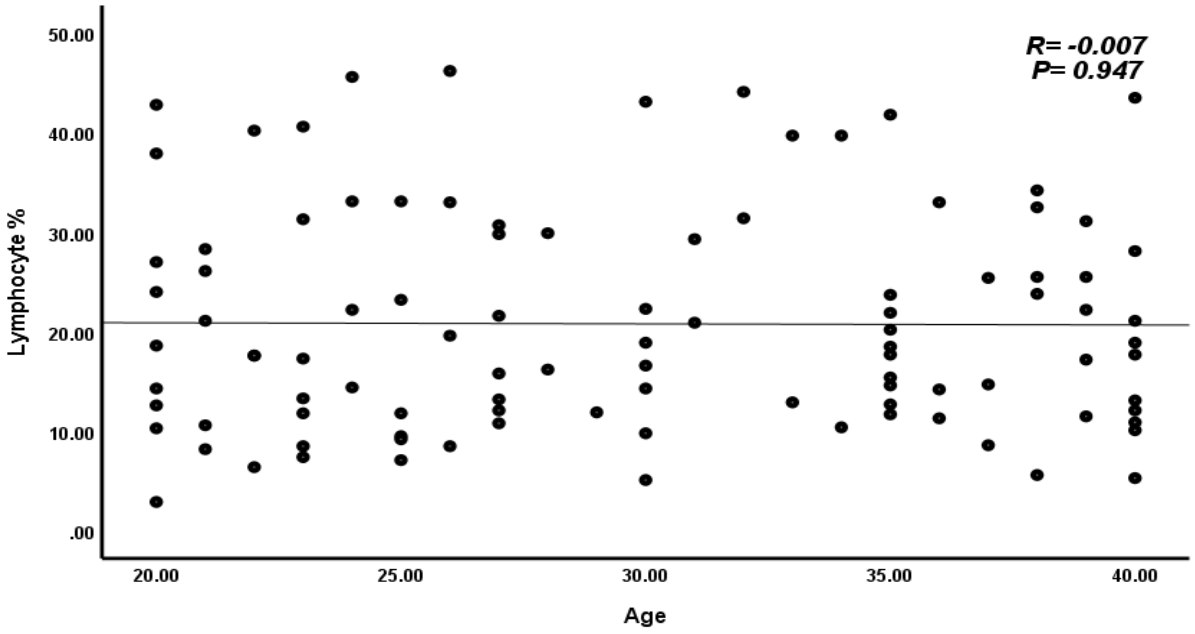

Figure 6: Correlation between lymphocyte % and age

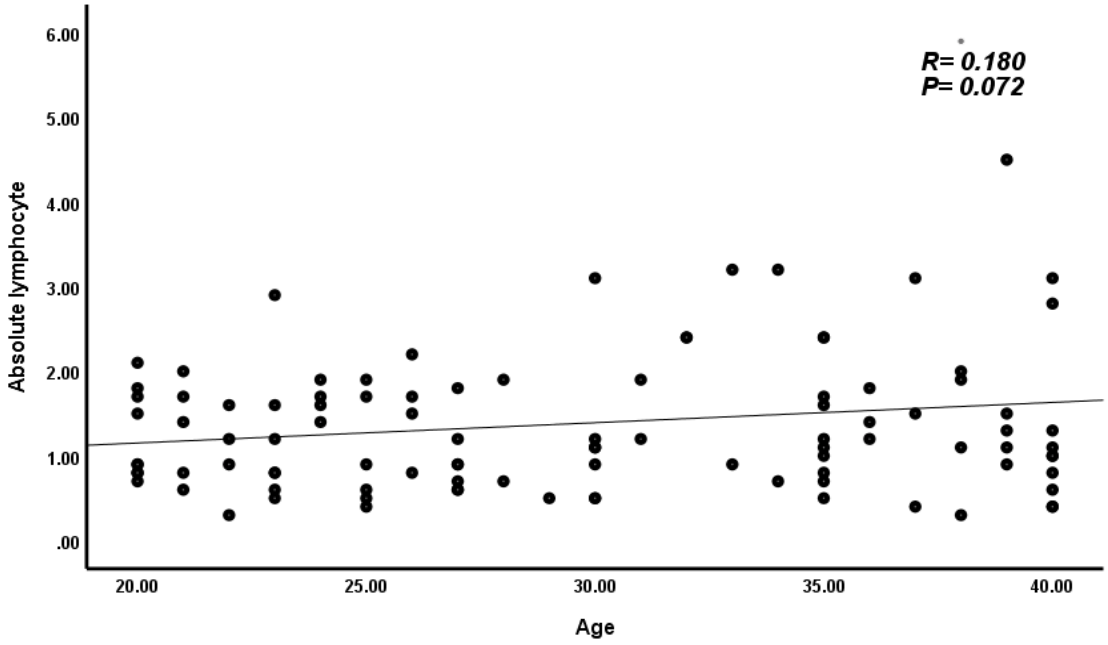

Figure 7: Correlation between absolute lymphocyte and age

Discussion:

This study showed that the TWBC count in malaria patients was within the reference values but it was lower than in control group with insignificant p-value ,previous study done by F.EllisMckenzie et al showed that wbc count from uninfected group and p.vivax and p.falciparum patients at the clinics in Thailand showed that the wbc count was lower in malaria patients(lower than 4000cell/ML) than uninfected group.[14]Lymphocytopenia was frequently seen in malaria infected group than non-infected group, about half of malaria patients have lymphocytopenia when measured in lymphocyte % and ALC, recent study report that Fas-induced apoptosis plays an important role in the lymphocytpenia of plasmodium falciparum malaria in human, the authors base their conclusion on concomitant findings of increased levels of soluble Fas ligand in serum.(Lars,2000,6089)

In this study the TWBC count was not affected by the different parasite densities, also no changes were seen in lymphocytes % and ALC between the two groups. A study done by ManasKotepui et al in Thailand between January 2009and January 2012 reported that the leukocytes count was significantly higher in patients with high parasitemia compared to those with low and moderate parasitemia and lymphocytes count was significantly lower in patients with high

parasitemia compared to those with low and moderate parasitemia groups (p0.0001).(ManasKotepui,2015,25) Another study done by Eze Evelyn M et al examined the experiential relationship between parasite density and haematological parameters in male patients with plasmodium falciparum infection in Port Harcourt, Nigeria found that inverse relation between lymphocyte count and parasite density suggested the increased apoptosis at higher parasitemia .(Eze Evelyn,2012,4) The R value in this study tested the correlation between lymphocytopenia and the different levels of parasitemia and reported a technically weak negative correlation ,the lymphocyte count showed slight reduction when compared between cases and control group, inspite of recording these counts within the normal range in both groups in different levels of parasitemia.

TWBC count and lymphocyte % were slightly reduced in females compared to males but with insignificant p-value, while they showed the same ALC in both gender. The R value showed a weak negative correlation.

The TWBC count and lymphocyte % were slightly reduced in relation to the advance in age. Correlation between lymphocytopenia and age showed weak positive correlation, P.value found to be not significant. A study done by Wilson L Mandala et al, in Malawa, found that lymphocytes subsets vary with age among Malawian children and that panlymphocytopenia is reported in children presenting with cerebral malaria(median lymphocytes count 2,100/ML) and uncomplicated malarial (3,700/ML)and finally, they stated that lymphocytopenia affected overall T and B lymphocytes subsets.(Wilson,2020)

The recent study showed high PPV (83%) which means that 83% of malaria patients whose test result is positive had lymphocytopenia , implying that false positive outcomes are minimized and moderate NPV with its greater proportion of false negative screening test. Another study found that a total 440 patients diagnosed with the malaria at the Harbour Hospital between January1999 and January 2012 with differential white cell count determined within the first 24hours, showed presence of lymphocytopenia in 52% of the all patients. Total lymphocytes count did not differ significantly between groups, analysis revealed a good negative predictive value but a poor positive predictive value of lymphocytopenia.(Joana,2018,21)

Conclusion :

This study concluded that there was nosignificantlymphocytopenia in malaria patients, and that lymphocytopenia cannot be used as the key haematological indicator of malaria infection. Moreover, lymphocytopenia has no relation with the level of hyperparasitemia.

More studies using larger number of subjects could be done, studying the relationship between the absolute lymphocyte count and the different plasmodium species.

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