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Research Article

PLACENTAL MALARIA IN HUMAN IMMUNODEFICIENCY VIRUS POSITIVE AND NEGATIVE PARTURIENT AT TERM: A COMPARATIVE CROSS -SECTIONAL STUDY

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Abstract

Background: The burden of malaria is severe in Africa, affecting mainly pregnant women and their unborn children. Malaria and Human Immunodeficiency Virus (HIV) combined account for 9% of the total burden of diseases in sub-Saharan Africa, with more than 80% of the related global deaths occurring in tropical Africa. **Objective**: We compared the proportion of placenta malaria in HIV positive and negative parturients at term and evaluated for possible associated factors. **Methods**: This was a prospective comparative cross-sectional study in which 90 HIV positive and 90 HIV negative pregnant women who at term had a full-thickness placenta biopsy, following delivery, for assessment of placenta malaria parasitemia. Their peripheral venous blood was also tested malaria parasite. Demographic and baseline variables were summarized using descriptive statistics. Comparison of continuous variables was done using student's t-test and categorical variables were compared using the Ch square or Fisher 'exact test as appropriate. 95% confidence interval and significant at reported at $p<0.05$. **Findings**: Placenta malaria was present in 11.1% of HIV positive women and in 21.1% of HIV negative women although all women in both groups were negative for malaria parasite on peripheral venous blood microscopy. Most of the positive pregnant women (80%) who had placenta malaria had previously been treated for malaria in pregnancy and this was about 10 times more (8.8%) than in women without placenta malaria (<0.001), thus was similar in HIV negative women. **Conclusion:** HIV positive pregnant women on HAART had about 2 -fold less prevalence of placenta malaria compare to HIV negative women. Both HIV positive and negative women who had been previously treated for malaria were significantly more likely to have placenta malaria compare to women who were not treated for malaria in pregnancy.

Keywords: Malaria, HIV.

INTRODUCTION

Africa is burdened with 95% global malaria and 96 % death, unfortunately, only four African countries are responsible for half of these deaths with Nigeria in the leading position (WHO, 2008). Pregnancy increases the risk of malaria by 2- to 3-fold and a quarter of the women in the holoendemic regions like Nigeria may be infected at the time of delivery (Otuli *et al.,* 2018). The risk of malaria in pregnancy is greater among younger pregnant women, women in their first or second pregnancies, first 2 months postpartum women with human immunodeficiency virus (HIV) infection and female migrants living in areas of low endemicity (Ayodele *et al.,* 2015; Clark, 2020; Mbachu *et al.,* 2020). Sub-Sahara Africa is a continent with more than its share of the burden of malaria and HIV infection in the world (Otuli *et al.,* 2018; Kwenti, 2018). These are worsened by other factors such as illiteracy, poverty, low socioeconomic state, poor government policies, geographical location and environment (Otuli *et al.,* 2018; Clark, 2020; Mbachu *et al.,* 2020; Kwenti, 2018). Malaria and HIV account for 9% of the total burden of diseases in sub-Saharan Africa, and a total of over 4 million deaths globally, of which more than 80% occur in Africa (Onankpa *et al.,* 2017). The outcomes of either of the two infections are more complicated by the coexistence of the other (Mbachu *et al.,* 2020; Kwenti, 2018). Malaria, co-infection with HIV is associated with increased complicated malaria enhanced failure and resistance to antimalaria drugs and congenital infection in neonates (Mbachu *et al.,* 2020; Kwenti, 2018; Onankpa *et al.,* 2017). Furthermore, HIV infection alters the typical gravidity-specific pattern of malaria risk by shifting the burden from first and second pregnancy to all pregnancies (Kwenti, 2018; Onankpa *et al.,* 2017). There is also the risk of adverse drug reaction if sulphadoxine-pyrimethamine and cotrimoxazole are used together for the prevention of malaria and opportunistic infection respectively in HIV positive pregnant women (Kwenti, 2018). In like manner, the dual infection causes mutation of the CCR5 leading to an increase in virus replications and a decline in CD4+ T-cell count (WHO, 2008; Kwenti, 2018). Thus hastening the progress of HIV to Acquired immunodeficiency disease (AIDS) (WHO , 2008; Kwenti, 2018; Onankpa *et al.,* 2017). The World Health Organisation (WHO) introduced a three-pronged approach to control malaria in pregnancy (WHO, 2008; Onankpa *et al.,* 2017). These include prompt case management, intermittent preventive treatment with sulphadoxine-pyrimethamine IPTp), and the use of insecticide-treated materials including insecticide-treated bed nets (ITN) (WHO, 2008). Unfortunately, case management is fraught with unreliable diagnosis and malaria is managed presumptively with the use of clinical symptoms leading to over-diagnosis, failure to diagnose and inadequate treatment. In addition, the parasites may be sequestered in the placenta even when no parasite was detected on microscopy of peripheral blood, microscopy diagnosis may not give an accurate estimate of the incidence of malaria (Ayodele *et al.,* 2015; Clark, 2020; Mbachu *et al.,* 2020; Ezeoke *et al.,* 2018). In this comparative study we evaluated for the presence of malaria parasites in the placenta

of HIV positive and negative pregnant women following delivery at term to determine its prevalence and associated factors.

METHODOLOGY

This study was a comparative cross-sectional study of HIV seropositive and seronegative parturients presenting for delivery at term at the department of Obstetrics and Gynaecology of LASUTH. The seropositive women were tested in accordance with the WHO serial testing algorithm and national algorithm. Inclusion criteria include consenting women who were booked, at term, with singleton pregnancy with no history of fever in the last two weeks before presentation. Excluded from the study were women with fever in the last two weeks preceding presentation at the clinic, and women with medical illnesses like renal disease, cardiac disease, liver disease, hypertension, diabetes and hemoglobinopathy or bleeding disorders. Patients with a history of blood transfusion in the last four weeks of pregnancy and women with AIDS complex or CD4 less than 200 cells/ml were also excluded.

Sample size calculation

The sample size for the study was calculated using the formula for a comparative cross sectional study (Karimollah Hajian-Tilaki, 2011).

$$n = \frac{2p\,(1-p)\,\{Z\alpha + Z\beta\}}{(p_1 - p_2)^2}$$

Sampling Technique: The results of the voluntary counselling and testing for HIV infection done at booking, was taken as evidence of HIV status of patients. HIV sero positive and sero negative parturients who met the selection criteria were consecutively recruited into the study. They were educated on the study and an informed consent form was signed. The desired sample size was attained using a convenient sample method. After recruitment into the study, each of the participants was assisted in filling a semi-structured interviewer-administered questionnaire. This included sociodemographic data, obstetrics history, history on sulphadoxine/pyrimethamine intake (IPT), malaria treatment in pregnancy and results of the research blood and placenta malaria tests.

Blood microscopy

Within two hours after delivery, 10mls of whole blood was collected from the women and was placed into an ethelene diamine tetraacetic acid (EDTA) bottle. The blood samples were received in the microbiology laboratory for malaria microscopy of Lagos State University Teaching Hospital (LASUTH) by a medical laboratory scientist who made both thick and thin blood films on the same slide for staining with 3% Giemsa solution. The thin blood film was used for parasite species while thick film for the malaria density.

Placenta histology

Three whole thickness placental biopsies were taken, one near the cord insertion and the other two at the periphery. The placental tissues were collected into universal bottles, preserved with 10% formaldehyde and sent to the histopathology laboratory of Lagos University Teaching Hospital (LUTH) for processing and analysis using Bulmer method (Bulmer *et al.,* 1993). The slides were then stained using Haematoxylin and Eosin (H & E) and Giemsa. The slides were read at Lagos University Teaching Hospital histopathology laboratory under the supervision of a consultant pathologist. A new two-parameter semi quantitative grading scheme that scores the degrees of inflammation and pigment deposition during placental malaria was adopted.

Ethical considerations

This study was approved by the health research and ethics committee of the Lagos State University Teaching Hospital, Ikeja. The participants were counselled on the research protocol in a language they understood. The participants, having been informed gave an informed consent. The study lasted over a period of seven months.

Statistical analysis

The data obtained was entered and analyzed using the Statistical Package for Social Sciences, (SPSS) version 19. Percentages, means and standard deviation of numerical variables were determined. Percentages of categorical data were also determined.. Means of numerical variables were compared using the Student's t-test, while the Chi-square test was used to test for association between categorical variables. Pearson and Spearman rho were used to find correlation between two numerical variables. Confidence interval was set at 95%. P-value level less than 0.05 was considered statistically significant.. Microsoft excel was used to draw charts.

RESULTS

A total of 180 parturients at term who met the selection criteria for the study were recruited. This was made up of 90 HIV positive parturients (Group A) and 90 HIV negative parturients (Group B). The age range of the studied population was 19 – 42 years while the mean age was 30.7 ± 4.8.

Table 1: Sociodemographic characteristics of participants by HIV status

Varaible	HIV status		x2	p
	Positive %	Negative %		
Age group				
Less than 25 years	13.3	8.9	3.600	0.165
25 – 34 years	60.0	73.3		
35 years and above	26.7	17.8		
Mean±SD	30.5±5.0	30.8±4.6		
Level of education				
Up to secondary education	64.4	35.5	15.02	<0.001
Tertiary education	35.6	64.4		
Occupation				
Skilled	15.6	50.0	34.63	<0.001
Semi skilled	58.9	28.9		
Unskilled	1.1	8.9		
Unemployed	24.4	12.2		
Religion				
Christianity	65.6	86.7	11.03	0.001
Islam	34.4	13.3		
Social class				
Upper	6.7	5.6	20.733	<0.001
Middle	28.9	62.2		
Lower	64.4	32.2		
Gravida				
Primigravidae	13.3	16.7	0.392	0.531
Multigravidae	86.7	83.3		
Parity				
Nulliparous	32.2	40.0	1.180	0.277
Multiparous	67.80	60.)		

Table 1 shows the socio-demographic characteristics of the participants based on their HIV status. The mean age of HIV positive women was 30.5±5.0, while that of the HIV negative women was 30.8±4.6, with a p-value 0.165. The differences in the level of education, occupation, religion and social class between the two groups were statistically significant with p-value <0.001.

Table 2. Determinants of placental malaria in HIV positive mothers

Variable	Malaria Histology diagnosis		
	Positive %	Negative %	p
Age group			
≤ 34 years	50.0	76.2	0.741x
>35 years	50.0	23.8	
Parity			
Nulliparous	30.0	32.5	1.000x
Multiparous	70.0	67.5	
Social class			
Upper	0.0	7.5	1.000x
Middle/Lower	100.0	92.5	
Parity			
Primiparous	30.0	32.5	1.000x
Multiparous	70.0	67.5	
Trimester at booking			
First/ Second	40.0	43.8	1.000x
Third	60.0	56.3	
ANC visits			
Less than four visit	10.0	3.80	0.381x
Four visits and above	90.0	96.3	
Doses of IPT			
1 – 2 doses	88.9	38.6	0.009x
3 – 4 dose	11.0	61.3	
Usage of ITN			
Occasional	66.7	60.7	1.000x
Often	38.3	39.3	
Malaria treatment in pregnancy			
Yes	80.0	8.8	<0.001
No	20.0	91.3	

Table 2 shows the summary of determinants of placental malaria in the HIV positive mothers using histological diagnosis. The number of doses of IPT and history of malaria treatment in pregnancy were significant associated with placenta malaria (p-<0.001). The determinants of placental malaria for the HIV negative group are summarized on [Table 3]. Trimester at booking and history of malaria treatment in pregnancy were significantly associated with diagnosis of malaria using placenta histology (with p-value < 0.05). When the analysis for determinants of placental malaria was based on the total population without first dividing the total population into HIV positive or HIV negative sub-sets, exactly the same variables were found to be statistically significant [Table 4].

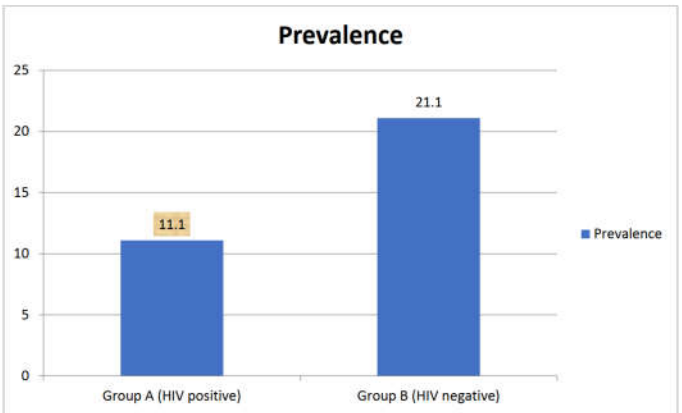

Figure 1. Prevalence of placental malaria using histological diagnosis

Figure 1 shows the prevalence of placental malaria using histological diagnosis among the two groups. Regarding the doses of IPT used in pregnancy, only one parturient who used more than two doses was positive, while 28 women who had less than three doses were positive. Interestingly the HIV status of the participants was not statistically significant. The HIV positive participants had more antennal visit 98% compared to 88% in the HIV negative women [Figure2] Some of the micrographs taken from the placental histology are shown in Appendix 1 to 4.

Table 3. Determinants of placenta malaria in HIV negative mothers

Varaible	Malaria Histology diagnosis		
	Positive %	Negative %	p
Age group			
Less than 34 years	78.9	83.1	0.738x
35 years and above	21.1	16.9	
Parity			
Nulliparous	47.4	38.0	0.460
Multiparous	52.6	62.0	
Social class			
Upper	10.5	4.2	0.284x
Middle/lower	89.5	95.8	
Parity			
Nulliparous	47.4	38.0	0.460
Multiparous	52.60	62.0	
Trimester at booking			
First/ second	47.4	71.8	0.044
Third	52.6	28.2	
ANC visits			
Less than 4 visits	21.1	8.5	0.209x
4 visits and above	78.9	91.5	
Number of IPT doses			
1 – 2 doses	100.0	92	1.000x
3 – 4	0.0	7.6	
Use of ITN			
Occasional/ rarely	75.0	47.7	0.252x
Often	25.0	52.3	
Malaria treatment in pregnancy			
Yes	78.9	18.3	<0.001
No	21.1	81.7	

Table 4. Relationship between HIV status and some variables

Variable	HIV positive%	HIV negative %	x2	P
Usage of SP				
Yes	98.9	83.3	13.445	<0.001
No	1.1	16.7		
Usage if ITN				
Ocassional / rarely	60.9	51.9	0.95	0.330
Often	39.1	48.1		
Febrile illness				
Yes	16.7	31.1	5.164	0.023
No	783.3	68.9		
Malaria treatment				
Yes	16.7	31.1	5.164	0.023
No	83.3	68.9		

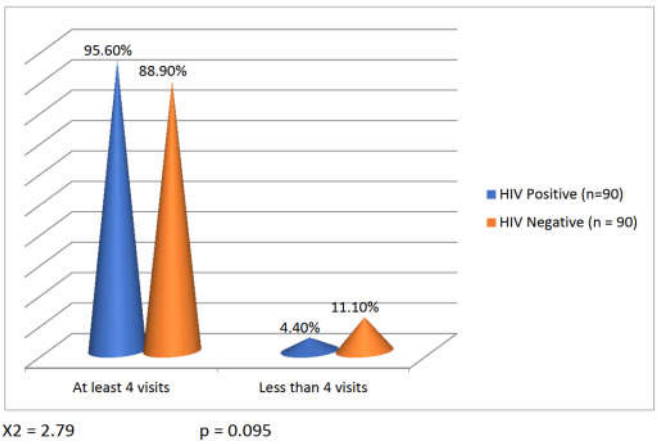

Figure 2. Number of ANC visits by HIV status of participants

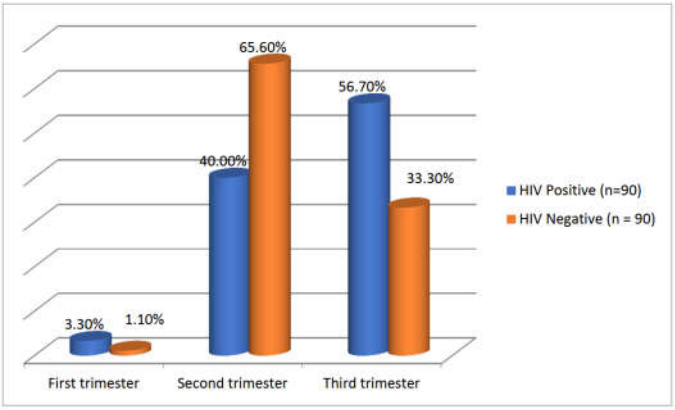

Figure 3. Trimester at booking by HIV status. if they booked more at third trimester , why more ANC visits let remove contradicts above

DISCUSSION

This study examined the prevalence, determinants and pattern of placental malaria using placenta histology in HIV positive and HIV negative parturients at term. The prevalence rate of placental malaria using placental histology in the 180 booked parturients at term at the Lagos State University Teaching Hospital was 16.1%. The prevalence of placental malaria parasitemia in urban and peri-urban settings in Africa varied from 1·6 to 69·6% (Ayodele *et al.,* 2015; Mbachu *et al.,* 2020; CISSE *et al.,* 2016; Oweisi *et al.,* 2018). In Sudan and Tanzania, the placenta malaria parasite was 32%and 75.5% (Ezebialu *et al.,* 2012). This marked difference in prevalence rate may be due to the method of diagnosis, age, socio-economic status, the density of the parasite, season, environmental differences and rate of transmission (Ezeoke *et al.,* 2018; CISSE *et al.,* 2016; Oweisi *et al.,* 2018; Ezebialu *et al.,* 2012). Placenta malaria is the presence of parasites in the intervillous space of the syncytiotrophoblast of the placenta (Clark, 2020). The parasite using the VAR2CSA gene binds the chondroitin sulphate, a proteoglycans in the intervillous space of the placenta (Ayodele *et al.,* 2015; Clark, 2020). Studies have shown parasitemia using the placenta tissue for histology which has been established as the gold standard for detecting malaria parasites in pregnancy (Clark, 2020; Ezebialu *et al.,* 2012). The prevalence rate of placental malaria using histological diagnosis in HIV-positive parturients in this study was 11.1% compared to a rate of 21.1% in the HIV-negative group, FIG 1. Similarly, a study in Uganda revealed a lower placental malaria prevalence among HIV positive population, than HIV negative population (Newman *et al.,* 2009). This pattern in our study could be because the result was obtained when 62% of the HIV positive population received abbreviated mono- or dual anti-retroviral therapy (ARV)with zidovudine, stavudine, zidovudine/ lamivudine or stavudine/lamivudine for the prevention of mother-to-child transmission of HIV (PMTCT),. A study showed that HIV-positive mothers who did not receive ARV had more placental malaria than those who did (Ezebialu *et al.,* 2012). Furthermore, a Ugandan study reported a malarial prevalence rate of only 4% among HIV-positive patients who were on HAART and who were asymptomatic for malaria, a scenario which is similar to this present study (Nakanjako *et al.,* 2011). ARV drugs boost the immunity by elevating the CD4- positive cells in HIV-positive women (Ezebialu *et al.,* 2012). Recent information suggests that HAART especially the protease inhibitors, lamivudine and nevirapine have a negative effect on Plasmodium specie and may have anti-malarial properties (Greenhalgh *et al.,* 2018; Anyanwu *et al.,* 2020). Moreover, the mean CD4 cells count in the HIV positive group in this study was 400 cells per ml. It was therefore not surprising that they have fewer episodes of malaria and fever 16.7% compared to 31.1% in the HIV-negative group. Onankpa et al like many other studies discovered that malarial parasitemia is observed to be more common in HIVpositive patients, especially those with low CD4+ counts with a consequent higher rate of clinical malaria (Onankpa *et al.,* 2017). Previous studies have also had been at variance with these findings consistently documenting a higher prevalence rate of malaria in HIV patients when compared with HIV negative patients.[5,6,8] Ezeoke et al in Southwest Nigeria using microscopy showed a higher malaria rate in HIV positive when compared to HIV negative women may be because the study size was larger compared to our study (Ezeoke *et al.,* 2018). IPT prophylaxis also has been proven to reduce malaria transmission in HIV-positive patients (Ayodele *et al.,* 2015; Ezeoke *et al.,* 2018; Ezebialu *et al.,* 2012). Apart from the possible suppressive effect of HAART on malaria, the HIV-positive parturients in this study were more likely to use IPT and ITNs. It was therefore not surprising that they have fewer episodes of malaria and fever 16.7% compared to 31.1% in the HIV-negative group. Malaria microscopy was not included in the final analysis despite being one of the diagnostic methods proposed for this study because all the results from microscopy were negative for malaria parasites (Mbachu *et al.,* 2020).

The fact that all the participants were asymptomatic for malaria may indicate that there was no acute malaria incidence, thus they were truly negative or they may be falsely negative due to sequestration of the parasite in the placenta. Apart from these reasons, microscopy itself may have lower efficacy in diagnosing malaria when compared with other methods. The absence of peripheral parasitemia does not exclude parasitemia, has low values my remains undetectable during microscopy due to low density (Clark, 2020; Mbachu *et al.,* 2020). This placental infection can occur in the absence of clinical symptoms or negative microscopy using peripheral blood or placenta blood (Clark, 2020; Mbachu *et al.,* 2020). However, parasites have a longer duration in the placenta compared to peripheral malaria parasites detected on microscopy (Mbachu *et al.,* 2020; Newman *et al.,* 2009). Age, parity and social class were not significantly associated with placental malaria histology in this study in keeping with previous findings by Ezeabuli *et al.* (2012) but in variance to okoli *et al.* (2014). This may be due to the fact that all the pregnant women in this study were booked at the teaching hospital and therefore had access to specialist care irrespective of their age, parity or social class (Ezeabuli *et al.* (2012). There is also a high rate of IPT use, which may have modified the effect of these variables.

Conclusion

Placenta malaria definitely ascertain a present or past parasitemia. Malaria and HIV infection have a mutual association and negative impact n the life of the mother and child. It is therefore essential to harness evidence based solution in the management of the dual infection

Limitation: This is a hospital based study and may not be a true reflection of the general population. Further studies in the community and multi-centre studies need to be conducted so as

to further understand the relationship between malaria and HIV infection in pregnancy.

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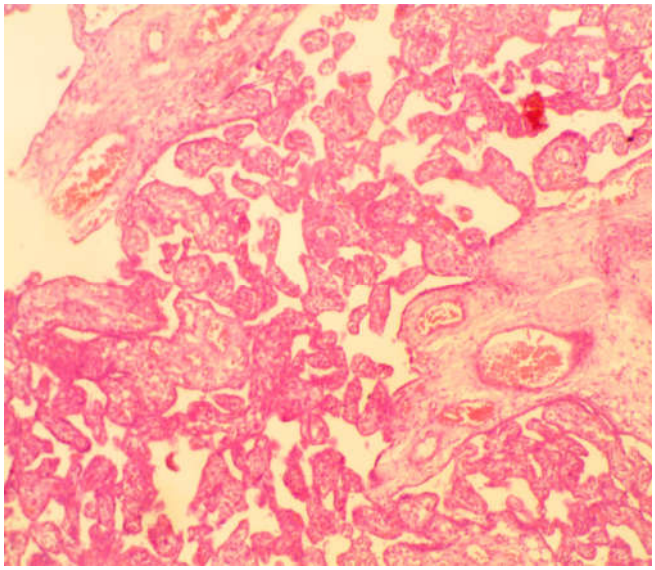
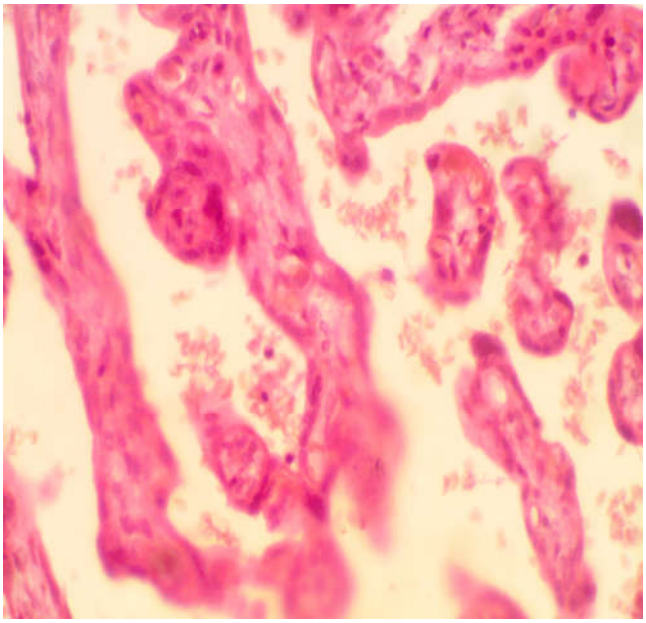
APPENDIX

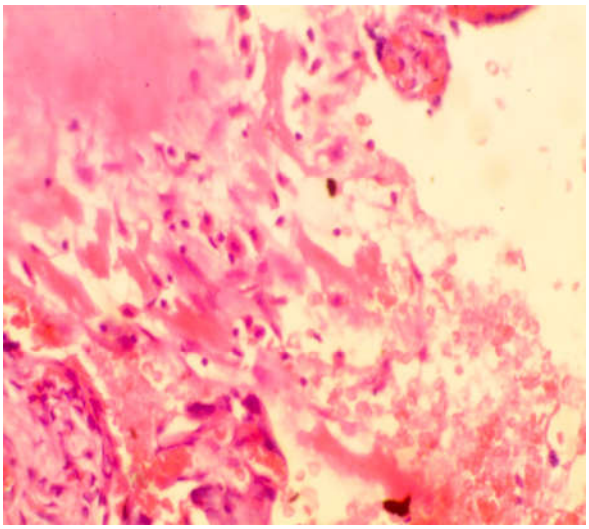
Appendix 1. Micrograph of normal placental histology (H & E slide), showing normal architecture without inflammation



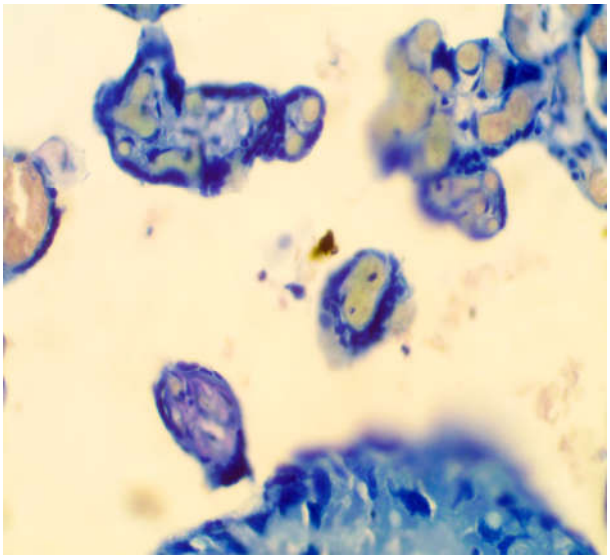
Appendix 2. Micrograph showing inflammatory score of I



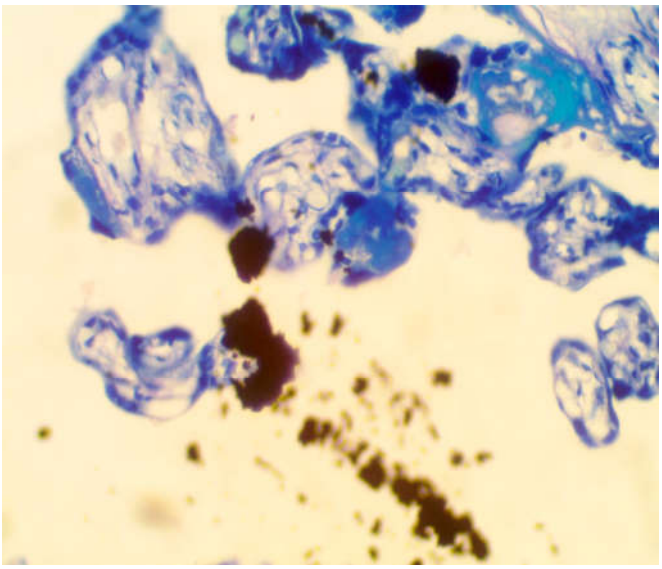
Appendix 3. Micrograph showing inflammatory score II

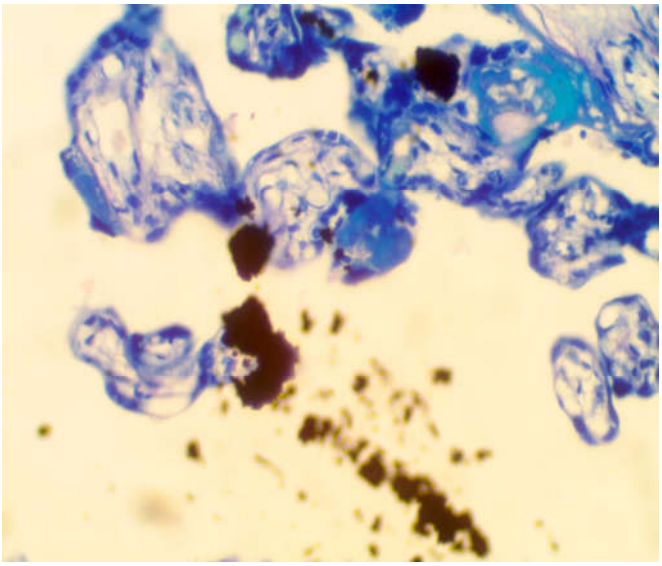


Appendix 4. Micrograph showing pigment score I



Appendix 5. Micrograph showing pigment score II





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