A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE PLACENTA IN MALARIA

by

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A HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE PLACENTA IN MALARIA

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE IN ANATOMICAL PATHOLOGY OF THE UNIVERSITY OF DAR ES SALAAM, 1991

I HAVE READ THE DISSERTATION AND APPROVE IT FOR THE EXAMINATION

SIGNED .....

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DECLARATION

I hereby declare that this dissertation has not been submitted for a degree award in any other University.

Signature .....

Date 1/10/91

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ACKNOWLEDGEMENTS

I am grateful to Prof. J. Kitinya for his supervision and constructive criticisms during the preparation of this dissertation. I am gratefully thankful for the collaborative research cooperation between the departments of histopathology at Muhimbili (Prof. J. Kitinya) and Immunopathology at Karolinska Hospital, Sweden (Prof. P. Biberfeld) through SAREC and WORLDLAB support for making it possible for me to obtain the antibodies used in this study. I am indebted to Dr. E. Kaaya for his technical advice on Immunohistochemical staining. I am thankful to Dr. H. Mwakyoma for his support by way of literature procurement. Mr. E. Chiragwire and Ms A. Magogo for their laboratory assistance, Ms. M. John for her secretarial services, Prof. H. Mgaya and nurses in ward 34 for their cooperation during data collection. I would like to thank all members of staff of the department of Morbid Anatomy and Histopathology for their moral support.

Lastly but not least I extend my gratitude to my husband Mr. Zebadiah S. Moshi and my sons Naiman and Nathan, who gave me moral support and suffered the long years of study I had to spend away from them.

ABSTRACT

Malaria is a highly prevalent infection in many developing countries and pregnant women are exposed to its ravages. Failure of other control measures and in view of a vaccine for malaria on the making, an understanding of the effects of malaria, especially its pathogenesis on the placenta is desirable.

Eighty-seven pregnant women were studied. 41 (47%) had malaria infections and 14 (16%) neonates had congenital malaria. There were 13 (31%) mothers who had preterm deliveries and had malaria. No statistical significance was found between preterm malaria infected and non-infected mothers.

Primigravid mothers had higher rates of infection compared to the multigravid. This difference was statistically significant. Low birth weight neonates were found to be more among the non-malaria infected mothers (60.9%). The mean weight differences for sixty-two term placentas with and without malaria infection was not statistically significant.

Eighteen placental sections were stained with Haematoxyline and Eosine stain, periodic acid silver methamine stain, antibodies for Pan leukocyte (CD45), B (CD45RA), T (CD45RO) cells and for collagen IV. Malaria parasites and pigments were found in the maternal intervillous space and fibrinoid necrosis was found to be more prevalent in the heavily infected placentas.

Thickening of the trophoblastic and foetal blood vessel basement membrane, a decrease in pan leukocyte, B cell and T cells infiltrates were also seen in the heavily infected placentas.

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INTRODUCTION

Malaria is derived from the word malaria meaning bad-air.[30] It is caused by a Protozoan *Plasmodium. P. falciparum, P. vivax, P. ovale* and *P. malariae* are the various species that infect man. Malaria is transmitted from man to man by the bite of a female Anopheles mosquito, but it can be transmitted by inoculation, as in blood transfusion or in drug addicts who borrow needles and syringes from each other without sterilizing them. Malaria infection is characterized by general malaise, fever, rigours and chills, when severe, cerebral malaria and algid malaria, hepatorenal syndrome, pulmonary oedema and splenic rupture are observed.[30]

Several methods have been used to estimate the malaria prevalence in a community. (i) The spleen rate; that is the proportion of children (aged two to ten years) in a community with enlarged spleens is used. From this the World Health Organization (WHO) has proposed the following classification.[30]

(1) Hypoendemic malaria with spleen rate 0-10%

(2) Mesoendemic malaria with spleen rate 11-50%

(3) Hyperendemic malaria with spleen rate consistently over 50%. The spleen rate in adults is also high.

(4) Holoendemic malaria with spleen rate consistently over 75%. The spleen rate in adults is low. It is this type of endemicity that the strongest adult tolerance is found.

(ii) Parasite rate (the proportion of the population in which malaria parasites are found) and (iii) Parasite count where parasite in blood can be counted against leukocytes or estimating the exact amount of blood examined,[30] are some of the parameters used for estimating malaria prevalence.

Efforts for malaria eradication have been made, but only in 1950's did WHO endorse the concept of malaria eradication. Chemotherapy and insecticides were the main methods of eradication and they proved successful in North America and Europe.[50] In Sub-Saharan Africa malaria still persists and is taking its toll.

In Tanzania, malaria is distributed in all regions of the country with a prevalence ranging from hyperendemic and holoendemic in two-thirds of the country (Kihamia 1983, unpublished report). Previously malaria was mainly found in lowlands especially along the coast but now high numbers of cases are also observed in the high altitudes of Iringa, Arusha, Tanga and Kilimanjaro. Although transmission occurs in most areas of the country throughout the year, the highest intensity is seen following the rainy season and it is influenced by humidity and temperature with the low lying coastal areas having the heaviest occurrence of the disease (Dahoma 1983, unpublished report). Malaria is probably the most wide spread infection causing considerable morbidity and mortality in Tanzania. In an "Evaluation of Health Sector" report of the Ministry of Health (1978), malaria was responsible for 9-13% of the diseases diagnosed in outpatient hospitals in Tanzania and as a cause of death malaria claimed 4-8% in a ten year period of 1967-197750. Among the top ten conditions causing a heavy burden to the health sector in a joint Swedish/Tanzania Health Sector review (1982) malaria as a single disease entity ranked second to pregnancy and its complications.[50]

Malaria is still a major public health problem, in Tanzania and the world, especially in developing countries' Preventive measures in pregnant women, especially chemosuppression seems inadequate.[50]

In view of malaria vaccine on the making,[47] a detailed understanding of the effect of malaria especially during pregnancy is desirable. It will also help in elucidating the pathogenesis of malaria and possibly its control.

In this study, a histological and immunohistochemical study of malaria infected placentas was done.

LITERATURE REVIEW

During pregnancy women are particularly exposed to the risk of acute malarial attacks. The prevalence and intensity as well as the frequency of its occurrence are increased by four to twelve times that of their non pregnant women counterparts.[7] The risk is high, not only to the non-immune entering endemic areas but even for the residents of endemic areas.[7] This increased risk is particularly high during the second trimester (24 weeks) and primiparas are proportionately more susceptible.[7]

The increased risk of malaria in pregnancy is multifactorial. The pregnancy state appears to reduce the immune status of the host therefore making her own malaria worse.[34,42,43] The presence of the placenta probably offers increased opportunity for infected erythrocytes to sequestrate in fairly dense numbers, a process which allows the development of erythrocytic schizogonic stages which exposes the host to the risk of releasing large numbers of erythrocytic merozoites into the circulation and the attendant risk of acute malaria attacks within relatively short periods.

The adverse effects caused by malaria to the pregnant women and foetus has been documented by many authors. They include anaemia (haemolytic, depressed erythropoiesis), cerebral malaria, abortion, premature labour and low birth weight.[14,18,26,29,32-34]

The last three conditions have been associated with damage to the placenta by the heavy placental infiltration by malaria parasites.[9,25]

The extreme rarity of congenital malaria[33,34,46] in new born infants in areas where the disease is highly endemic is against congenital malaria as a possible cause of low birth weight in such infants, although contrary views have been expressed.[1,35]

An immune response to malaria has been demonstrated by the presence of hyperplasia of reticuloendothelial system[17,19] and by the fact that peak prevalence for malaria infection in pregnancy has been found in primigravidae33,34 with recovery of both primigravidae and multipara during late pregnancy.[5,34]

Cellular accumulation of polymorphonuclear cells and monocytes in the liver and thymus

in experimental malaria in mice suggest an immunological reaction.[52] In cerebral lesions which included lifting of endothelial cells from the basement membrane associated with the presence of monocytes, erythrocytes and parasites also suggest an immune response.[51] Further evidence for an immune response in malaria is shown by the release of Tumour Necrosis Factor (TNF),[45] the activation of T-lymphocytes[39,44] and macrophage infiltration.

In some experimental studies, TNF (cachectine), lymphocyte activating factor and Type I interferon have been implicated,[11,12,48] and some of these are also thought to account for certain unexplained pathological features in human malaria such as glucocorticoid antagonism and bone marrow suppression. Specific humoural responses where IgG and IgM antibodies develop after infection have been observed; but studies where IgG preparations have failed to exert a growth inhibition effect on the parasite have been reported,[51] suggesting that other factors may be responsible for the inhibition of parasite growth.

Information concerning placental pathology in malaria is scanty, especially in East Africa.[26] The accumulation of masses of infected red cells in malarial placentas,[3,4,26] with macrophages and an intense lymphocytic reaction have been documented.

A combination of parasitaemia, macrophage infiltration, malaria pigment deposits, syncytiotrophoblastic necrosis and trophoblastic membrane thickening reported in most studies[16a,18,30,53] may represent the form of tissue injury that lead to adverse effects of malaria on the foetus and that these are probably tissue reactions to immune processes stimulated in the host by malaria organisms.[18]

OBJECTIVES

Histological and immunohistochemical study of malaria infected placentas was done.

1. Histological examination of malaria infected placentas using: Haematoxylin and Eosin and periodic acid with methamine silver stains.

2. Immunohistochemical study of malaria infected placentas using the double indirect immunoperoxidase method for Pan leukocytes, B and T lymphocytes and collagen IV fibres were stained using CD45, CD45RA, CD45RO and anti-collagen IV antibodies respectively.

3. Maternal parity, gestational age and weight of the neonates were compared between mothers with and without malaria infection.

4. Weight differences were looked for between malaria and non malaria infected placentas.

MATERIALS AND METHODS

All placentas from indigenous African mothers residing in Dar es Salaam who delivered at Muhimbili Medical Centre between October to December 1990 whose blood slide were available were collected. These mothers were of gestational age between 28-42 weeks and were either referred or self referred. Information on their parity and last normal menstrual period was obtained. Where it was not possible, the fundal height was used as an estimate for the gestational age. Before or during labour a finger prick on the mother was made and a thick smear was obtained. After delivery, the infant was weighed, and its sex recorded. A cord blood smear was taken from the cut end after wiping off the Wharton's jelly and another smear was made by wiping the placental surface (maternal) off blood and forming a small cut from which the smear was taken. These slides were air dried then stained with Giemsa stain. They were examined with a light microscope under oil immersion for the presence of malaria parasites which was taken as evidence for malaria infection.

The placentas were fixed in cold neutral phosphate buffered formalin (pH7) in order to avoid formation of formalin pigments. Before weighing the placentas, blood clots were removed, its membranes and a standard cord of 30cm long was included. The diameter and thickness of the placenta were measured to the nearest centimeter.

The sections of the placentas were processed and embedded in paraffin in the usual manner. 4 micron thick sections were stained with Haematoxylin and Eosin. Periodic acid and silver methamine staining was carried out as follows:-[59]

The placenta and kidney section (used as positive control) were immersed in chloroform for fifteen minutes then in alcohol series of decreasing concentration and in deionized water. They were then put in 1% periodic acid for ten minutes, flushed in distilled water for several seconds and applied silver nitrate for one and half hours at 60 degrees centigrade. Before proceeding further by microscopic control the glomeruli should appear black. The sections were then flushed in deionized water for several seconds followed by Gold chloride (0.25%) for 30 seconds washed with deionized water for a few seconds, then 3% Sodium thiosulphate for 2 minutes. Washed in water, dehydrated, cleaned and

mounted in DPX. They were then examined with light microscope.

Eighteen cases were chosen for immunohistochemical studies. These were cases whose placenta was histologically found to have malaria parasites.

For immunohistochemistry, the paraffin sections were deparaffinized by incubating them in an air oven at 60^{o}C for fifty minutes followed by xylene at the same temperature for ten minutes then xylene at room temperature for ten minutes. They were then rehydrated to 70% alcohol.

Double indirect immunoperoxidase staining was carried out according to Naish[38] as follows: Sections were incubated in 5% hydrogen peroxide in methanol for twenty-five minutes to block endogenous peroxidase. The slides were washed for twenty minutes in Tris-Buffered Saline (TBS). Cross reacting antigens were blocked by incubating them in 1% Normal human serum in Bovine serum albumin and TBS (NHS/BSA/TBS) for twenty minutes. Excess serum was wiped off and the primary monoclonal antibodies, were applied overnight at 40 degrees centigrade (Table 8), since the tissues were formalin fixed and paraffin embedded, enzymatic predigestion with protease type XIV (Sigma Chemical Co., Dorset England) was used for collagen IV staining before application of the primary antibody.[38] Secondary antibodies were applied and incubated for 20-30 minutes at room temperature.

3,3'-diaminobenzidine tetrahydrochloride (DAB) with hydrogen peroxide was used as the chromogenic substrate. After obtaining the optimal brown colouration they were immediately rinsed in water and counterstained in Harris-Haematoxylin and mounted in DPX.

For positive controls, human lymphnodes were stained simultaneously with placenta sections while for negative controls TBS instead of the primary antibodies were used.

The results were semi-quantitatively categorized as follows:-

(a) Concentration of malaria parasites in placental sections.

1. 0-9 malaria parasites/HPF = + (mild)
2. 10-20 malaria parasites/HPF = + + (moderate)
3. > 20 malaria parasites/HPF = + + + (heavy).

(b) The concentration of immunohistochemically positive stained cells.

1. 0-9 positive cells/HPF = + (few)
2. 10-20 positive cells/HPF = ++ (moderate)
3. > 20 positive cells/HPF = +++ (abundant).

(c) For anti-collagen IV staining, the outcome was categorized as either:-

1. Normal : If linear continuous fibres where observed
2. Thickened : If there was duplication and discontinuous collagen fibres.

The staining intensity in all immunostaining categories were observed and recorded. Where necessary Chi-square test and Wilcoxon-rank sum test2 was used at P= 0.05 as level of significance.

RESULTS

A total of eighty-seven mothers of gestational age 28-42 weeks were studied (Table 1). Of these 41 (47%) had malaria parasites. 9 (22%) were found in the maternal finger prick smears, 18 (43.9%) in placenta smears and 14 (34.1%) in cord blood smear. (Table 2) indicating that there were 14 (16%) neonates with congenital malaria. Of the 87 mothers 25 (28.7%) were preterm and 62 (71.3%) were term deliveries. Among the malaria infected mothers 13 (31.7%) were preterm while 12 (26%) were preterm in the non infected mothers (Table 3). This difference was not statistically significant. Parity ranged from primiparous 35 (40%) to grand-multiparous 26 (30%) (Table 4).

Malaria infected mothers of low parity (primiparous) were more 21 (51%) compared to grand multiparous 7 (17%) and others 13 (31.7%). This difference was statistically significant ($P < 0.02$) (Table 5).

The number of low birth weight neonates delivered by non-malaria infected mothers was 28 (60.9%) compared to 15 (36.6%) in malaria infected mothers (Table 6).

There were eleven term placentas with malaria parasites whose mean weight was 568, S.D 118, whereas 51 term placentas without malaria parasites had a mean weight of 518, S.D. 150 (Table 7). This difference was not statistically significant. For the 18 cases chosen for immunohistochemical study, the following were observed.

Histology

In the non malaria infected placentas, inflammatory cells were not observed. Few fibrinoid necrotic villi were observed in term placentas. Pigments were found in these areas. 12 (66.6%) were mildly infected with malaria parasites 4 (22.2%) were severely infected and 2 (11%) were moderately infected (Table 9).

All the mildly infected placentas had parasites limited to the intervillous space. No parasites were found in the foetal circulation. Malaria pigment had the same distribution as that of the malaria parasites and was also present in macrophages. Fibrinoid necrosis involved the whole or part of villi. Inflammatory cells included lymphocytes, macrophages and polymorphonuclear cells (PML). In the mildly infected cases lymphocytes were found

in all whereas macrophages were observed in 6 (33.3%) cases. Most of the macrophages, contained malaria parasites, pigments and red blood cell debri. PML were found in two cases.

Among the heavily(4) infected placentas, both the malaria parasites and pigment were limited to the intervillous space. Fewer macrophages containing ingested malaria parasites, pigments and erythrocyte debri and lymphocytes were found. PML were not seen. Fibrinoid necrosis involving partial or complete villi was abundant and sometimes confluent in these heavily infected placentas.

The moderately infected placentas (2) had similar features as the heavily infected placentas except that the lymphocytes were moderate in number. PML were not observed.

In Periodic Acid and Silver Methamine Stain (PASM)

Fifteen cases were available for PASM Stain. Brown to black silver deposits were seen in the trophoblastic and foetal blood vessel basement membrane.

Thick and multiple linear deposits on the basement membranes were observed in varying degrees in the heavily infected placentas.

Immunohistochemistry

(i) **CD45** (A marker for pan Leukocyte) (Table 10)

Seventeen cases stained positively for CD45 showed a brown granular reaction product of uniform intensity which was seen in the cytoplasm and on the cell surface. The concentration of the CD45 positive cells varied from few to abundant.

Among the mildly (12) infected placentas, 9 had few positive cells, one abundant and two moderate. In the heavily (4) infected cases, two had few positive cells one moderate and one case did not show any CD45 positive cells.

The moderately infected placentas (2) had abundant positively stained cells.

(ii) **CD 45 RA** (A marker for B lymphocytes) (Table 10)

Thirteen out of the 18 infected placentas showed a brown granular reaction product on the surface and in the cytoplasm of the cells.

In the mildly infected placentas, one case had abundant positively stained cells. It was the same case with abundant CD45 positively stained cells. Two cases did not show any positively stained cells and one was inconclusive The remaining had few positive cells. For the heavily infected placentas two cases had few positive cells and the other two were negative.

The moderately infected placentas had moderate to abundant positive cells.

Staining intensity was the same in all cases.

(iii) CD45RO (A marker for T lymphocytes) (Table 10)

Twelve infected placentas stained positively for CD45RO- showing a brown granular reaction product on the cell surface and in the cytoplasm. Five were negatively stained and one case was inconclusive.

Among the mildly infected placentas, eight had few positively stained cells and three were negative. The heavily infected placentas had few positive cells in two cases and the remaining two were negative. Few positive cells were seen in the moderately infected placentas. Staining intensity was the same in all cases.

(iv) Anti-collagen IV (Table 11)

Twelve placentas were available for anticollagen IV staining.

Brown reaction product was seen clearly along the foetal blood vessel basement membrane of the villi. Few villi with discontinuous staining were observed on the maternal basement membrane immediately below the trophoblastic cells.

Staining intensity varied. It was mainly weak in the heavily infected placentas, which showed discontinuous, non-linear and patchy reaction product along the collagen fibers. This was observed in 10 of the placentas with varying degrees. It was most pronounced in the heavily infected placentas and in villi which had undergone partial or total fibrinoid necrosis.

DISCUSSION

The prevalence of malaria in pregnancy during the period between October to December 1990 was (47%). In other studies the prevalence varied 43.9% and 20.2% in Gambia, 54% in Central Africa and 9.6% in the Southern Pacific.[18] In Tanzania, a seasonal variation was observed among individuals living in the rural group where it was at its peak immediately after the rainy season and lowest during the dry season.[26] This study was done immediately after the short rainy period. This could have contributed to the rather high rate.

Malaria parasite distribution varied, more were found in the placental blood smears (43.9%) followed by cord blood (34.1%) and lastly maternal peripheral blood (22%). The placenta has been found to concentrate malaria parasites so that one can have a negative peripheral blood smear with a positive placental smear.[33,34] The mechanism for this tendency is thought to be due to the sludging of the stickier parasitized red cells in the eddies of slow moving placental stream, in which high glucose content is found which favours the development of the parasite.[16b] The proportion of congenital malaria, that is the positive cord blood smears examined was 16% in this study, which is high. One report has documented several series in which the prevalence of congenital malaria varied from none to 21%.[29] A study done in Tanzania had a prevalence of 38%.[26] Lower parasitaemia levels have been observed when peripheral blood smears of the neonates are taken rather than the cord blood smears.as in this study. This may be due to early clamping of the cord during labour.[26] Of the 87 mothers in the present study 25 (28.7%) were preterm deliveries of whom 13 (31.7%) had malaria infection. This difference was however not statistically significant. There was a high proportion of malaria infected pregnant women with low parity (51% primigravidae) compared to grand multipara and others (17 and 31% respectively). This difference was statistically significant ($P < 0.02$). Several studies have associated low parity with peak malaria prevalence and have associated these findings with the involvement of the immune response.[1,33,34] The actual mechanism for this difference is not established but while Kortman has attributed this difference to age

dependent change in maternal immunological maturity,[26] McGregor[34] attributes this difference to gradual strengthening of cellular response to plasmodial antigens due to repeated infections in subsequent pregnancies which effectively limits the parasite. The latter seems more plausible. In Zaire,[31] age and parity did not constitute risk factors for a high malaria rate in pregnancy.

Low birth weight neonates delivered by non-malaria infected mothers were more (60.9%) compared to those of the malaria infected mothers. Other studies differed in that low birth weight neonates were found mainly in the malaria infected mothers.[18,26,29,32,33,34] However, a study in Ubangi found no consistent relationship between maternal anaemia, splenomegaly, hydramnios, Apgar score, birth weight, infant's length and head circumference. In fact the rate of hydramnios was found to be higher in non-malaria infected placentas and was concluded that mothers with falciparum malaria in Ubangi district (Zaire) delivered apparently unaffected infants.[1] Another study in Zaire showed low birth weights in newborns with malaria compared to those without malaria.[31] Different outcomes of low birth weight in neonates in malaria could be due to the fact that other conditions may cause low birth weight such as; maternal height which is a reflection of genetic and constitutional factors, nutritional factors such as gestational weight gain, general morbidity, toxic exposures such as cigarettes, alcohol consumption and social economic status[23,36] (Table 12) which were not considered in this and other studies.

Weight differences between placentas of infected and non infected mothers was not statistically significant in this study as well as in several others[31,40,41] but in one study, placentas with malaria were found to have significantly less weight than those with no malarial changes.[53]

In Haematoxylin and Eosin sections, malaria parasites and pigments and monocytes were found in between the villi. No malaria parasite were seen in the foetal circulation. Other studies have revealed similar findings.[18,53]

Fibrinoid necrosis was widespread in the heavily infected placentas than in mildly infected or non-infected placentas, although fibrinoid necrosis is a common feature in term

placentas.[27]

Mononuclear cells and macrophages were among the reactive cells found in this study. Only two cases had a PML infiltrate together with the mononuclear cells. PML have been found to phagocytose plasmodium infected red-blood cells to a greater extent than they phagocytose effete red blood cells.[10] This was greatly increased in immune sera while it was not increased in individuals recovering from a first attack of plasmodium falciparum infection, who presumably have no or little malaria antibodies or opsonisation. Thickening of the trophoblastic foetal blood vessel basement membrane as demonstrated by PASM stain and anticollagen IV staining in this study has also been demonstrated by Galbraith et al[18] and Walter et al[53] who used the Periodic acid Schiff's reaction and ultrastructural analysis. PASM staining was used in this study because it stains basement membranes better.[59] Transplacental transport of exchange of nutrients, antibodies and waste products from the foetus may be impaired by this thickening.

Positivity for CD45, CD45RA and CD45RO demonstrated the presence of Pan Leukocytes, B and T-cells respectively.

T-cell epitopes on the malaria parasite have been identified on both circumsporozoite and malaria protein gp 190.[13,20,24] However as shown in Table 10, an inverse relation in the concentration of B and T lymphocytes and the severity of malaria infection was found in this study. Studies demonstrating sub-population changes of lymphocytes have shown a reduction in the absolute number of circulating T-cells and a relative increase in the number of B-cells and null cells in malaria.[57]

These changes have been associated with a modified immune responsiveness[56,58] which comprised of immune suppression, lymphocyte polyclonal antibodies to T and B cells and soluble circulating malaria antigens leading to survival of the parasite. Suppression of humoural response by Plasmodium falciparum is mediated through adherent cells, especially macrophages and T-cells.[22,54] Immunosuppression of cell mediated response and suppression of lectin induced blastogenic response on lymphocytes occurred provided parasitaemia exceeded 25%49. For example, patients with cerebral malaria and others

with high parasitaemia without cerebral involvement had a diminished or negative response to candidin during the acute phase, and their blastogenic response to lectin was impaired whereas the proliferative response to candidin was suppressed in parallel with the delayed cutaneous response to the same antigen.[6,37] In one animal experiment, results on cell mediated immune suppression have been inconclusive.[21] Lymphocytotoxic antibodies have been demonstrated in the serum of patients with falciparum or vivax malaria.[28,54] These were of IgM class and were reactive against both T and B cells. Since IgM immunoglobulins do not cross the placenta,[55] the lymphocytotoxic effect on the T and B-cells due to this immunoglobulin is absent in the foetal circulation. It is possible therefore that this lack of cytotoxic antibody and the presence of other presumably protective antibodies from the mother in the foetus may contribute to the rarity of congenital malaria.

Macrophage dysfunction has been implicated as a cause of immunosuppression in malaria infection.[8,21] Macrophages have been found to produce a range of mediators of which Tumour Necrosis Factor(TNF), Lymphocyte Activating Factor (LAF) and Type I interferon are increased in acute malaria.[11] In invitro studies, supernatants from the phagocytic macrophages show that increasing concentrations of parasite material that is phagocytosed leads to supernormal concentrations of LAF, up to a certain limit when further increases in parasite material led to subnormal amounts of LAF secretion. This would indicate that alterations of the macrophage function was related to the amount of parasites ingested.[58]

It is possible therefore that the degree of immunosuppression in severe malaria infections of the placenta results in lower mononuclear infiltration as was found in this study.

SUMMARY

Malaria is probably the most widespread infection causing considerable morbidity in Tanzania and other developing countries.

There is an increased risk of malaria attack during pregnancy since the pregnancy state reduces the immune status of the host.

Efforts for its eradication, both chemotherapeutically or by insecticide use have not proved successful in developing countries. Because of the now possible development of a malaria vaccine, a better understanding of the effects of malaria on pregnancy is desirable.

A histological and immunohistochemical study of malaria infected placentas for possible pathology during the period of October to December 1990 was carried out. The following results were obtained:

Eighty-seven selected pregnant mothers of gestational age ranging from 28-42 weeks showed a malaria infection prevalence rate of 47%. The study was done after the rainy season, a period which is known for peak malaria prevalence, which may explain the high prevalence rate. Malaria parasites were found mainly in the placental blood smears. The prevalence of congenital malaria was also high (16%). 28.7% of the deliveries were preterm, 31.7% of the mothers were infected with malaria. No statistically significant difference was found between preterm malaria and non-malaria infected mothers.

There were statistically significant more malaria infected pregnant women with low parity (51%primigravidae) compared to multipara (17%) and others (31%). Most other studies done have shown similar findings. The number of low birth weight neonates in non malaria infected mothers was higher (60.9%) than those of malaria infected mothers. There have been inconsistent findings concerning birth weights and placental weight differences between malaria infected and non- malaria infected mothers and the effects of malaria on premature labour. There is a need therefore for well controlled studies that should include other risk factors for low birth weight and premature labour if a rational explanation for this malaria effect is to be formulated.

As in other studies, malaria parasites were found mainly in the maternal intervillous

spaces. No parasites were found in the placental foetal circulation. Fibrinoid necrosis was more widespread in the heavily parasitized placentas together with thickened foetal blood vessels and trophoblastic basement membrane as shown by PASM and anti collagen IV immunohistochemical stain.

Altered immune response in the heavily infected placentas was observed as shown by the presence of fewer numbers of panleukocytes, B and T-cells compared to non-infected or mildly infected placentas. This observed decrease in the immune response to malaria is demonstrable in pregnant mothers.

RECOMMENDATIONS

1. There is a need for controlled studies that will take into account other risk factors for low birth weight and premature labour, so as to have a rational explanation for the effect of malaria on low birth weight and premature labour.
2. There is a need for quantitative morphometric analysis of basement membrane changes between heavily and non or mildly infected placentas.
3. Pregnant mothers should be given priority for vaccination when a malaria vaccine is available.

Table 1: Gestational age distribution of the study population

Gestational Age (wks)	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total
Number	2	1	-	-	2	-	3	1	16	3	13	7	33	3	3	87
(%)	2.3	1.1	-	-	2.3	-	3.5	1.1	18.4	3.5	14.9	8.0	37.9	3.5	3.5	100

Table 2: Malaria parasite distribution in maternal, placental and cord blood smears

Site	No.	%
Maternal Peripheral blood	9	22
Placental blood	18	43.9
Cord blood	14	34.1
Total	41	100

Table 3: The distribution of term and preterm deliveries in the study population.

	Preterm	%	Term	%	Total
Malaria infected mothers	13*	31.7	28	68.3	41
Non Malaria infected mothers	12*	26.1	34	73.9	46
Total	25	28.7	62	71.3	87

KEY: 'Term' is taken as a baby born after 37 completed weeks of gestation.

* There was no statistical significance

Table 4: Frequency distribution of parity

Parity	1	2	3	4	5	6	7	8	9	10	Total
Number	35	10	11	5	7	5	4	4	4	2	87
%	40.3	11.5	12.6	5.8	8.0	5.8	4.6	4.6	4.6	2.3	100

Table 5: Distribution of parity among malaria infected mothers

	Primi-gravids	%	Grand multi-para	%	Others	%	Total
Malaria infected mothers	21*	51.2	7*	17.1	13*	31.7	41
Non Malaria infected mothers	14	30.4	19	41.3	13	28.3	46
Total	35	40	26	-	26	-	87

KEY: Grand multipara is a woman who has given birth 5 times or more.

* There was statistical significance $X2 = 6.6$ $P < 0.02$, (d f = 2).

Table 6: Distribution of birth weight among malaria and non-malaria infected mothers.

	Above 3rd centile	%	Below 3rd centile	%	Total
Malaria infected mothers	26	63.4	15	36.6	41
Non-malaria infected mothers	18	39.1	28	60.9	46
Total	44	50.6	43	49.4	87

KEY: Neonates below 3rd centile (Dubowitz) are regarded as being Low birth weight children.

Table 7: Distribution of weight in term placentas with and without malaria infection

		Weight (gm)	
	Number	Mean	SD
Term placentas infected with malaria	11	568	118
Term placentas not infected with malaria	51	518	150

There was no statistical significance: z = 0.32 (Wilcoxon rank sum test).

Table 8: Primary Antibodies used in the study

S/No	Antibody	CD Class	Specificity	Source	Dilution
1	LCA.T200	CD45	Common leucocyte antigen	DAKO	1:20
2	UCHL-1	CD45Ro	T cells	DAKO	1:20
3	F8-ll-13	CD45RA	B cells	DAKO	1:2000
4	Anti-Collagen IV	-	Collagen IV	DAKO	1:100

Table 9: Concentration of malaria parasites and inflammatory cells in the malaria infected Placentas.

Case	Conc of mps	Lymphocytes	Macrophage	Polymorphs
1	+ +	/	/	-
2	+	/	-	-
3	+	/	/	/
4	+ +	/	/	-
5	+	/	/	-
6	+	/	/	-
7	+	/	-	-
8	+	/	/	-
9	+	/	-	-
10	+	/	-	-
11	+	/	/	-
12	+	/	/	/
13	+	/	-	-
14	+ + +	/	-	-
15	+	/	-	-
16	+ + +	/	/	-
17	+ + +	/	-	
18	+ + +	/	-	-

KEY:
(1) 0-9 mps = + (mild)
(2) 10-20 mps = + + (moderate)
(3) > 20 mps = + + + (heavy).
(4) / = Present.
(5) - = absent

Table 10: Concentration of Pan leukocytes (CD45), B (CD45RA) and T cells (CD45RO) in malaria infected placentas.

Case no	CD45	CD45RA	CD45RO
1	+ + +	+ + +	+
2	+ +	+	+
3	+	+	+
4	+ + +	+ + +	+
5	+ +	-	?
6	+	+	-
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	+ + +	+ + +	+
12	+	-	-
13	+	?	-
14	-	-	-
15	+ +	+	+
16	+	+	+
17	+ +	+	-
18	+	-	+

KEY:

(1) 0-9 positive cells = + (few)
(2) 10-20 " " = + + (moderate)
(3) > 20 " " = + + + (abundant).

Table 11: Staining results with Anti collagen IV, on malaria infected placentas.

Case No.	Anti collagen IV
1	T
2	N
3	N
4	T
5	N
6	T
7	T
8	N
9	T
10	N
11	M
12	-
13	T
14	T
15	T
16	T
17	T
18	-

KEY:

T = Thickened : Duplication with discontinuous collagen fibres.

N = Normal : Linear continuous collagen fibres.

Table 12: Established determinants of intrauterine growth(37) (modified).

DIRECT

A. Genetic and Constitutional Factors

1. Infant sex
2. Racial/ethnic origin
3. Maternal height
4. Pre-pregnancy weight
5. Paternal height and weight
6. Maternal birth weight.

B. Obstetric factors

1. Parity
2. Prior lowbirth weight infant

C. Nutritional factors

1. Gestational weight gain
2. Caloric intake.

D. Maternal Morbidity During pregnancy

1. General morbidity
2. Malaria.

E. Toxic exposures

1. Cigarette smoking
2. Alcohol consumption
3. Tobacco chewing.

INDIRECT

F. Demographic and Psychosocial Factor

1. Maternal age
2. Socioeconomic status.

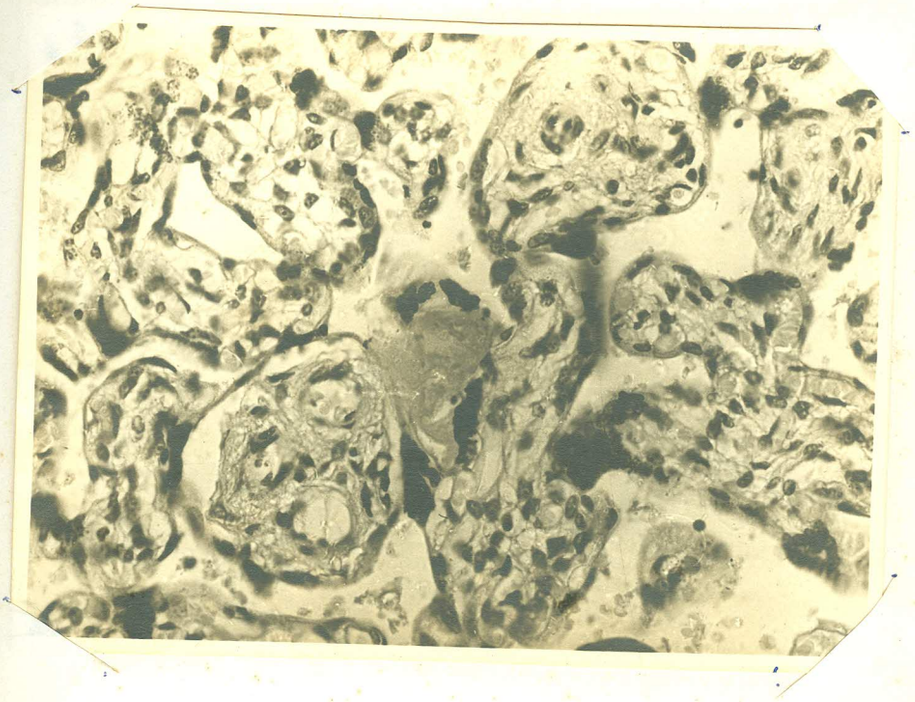


Figure 1: Section of placenta not infected with malaria. Haematoxylin and Eosin, Original magnification x400

Figure 2a: Section of placenta heavily infected with malaria. Note the numerous malaria parasites limited to the intervillous space (arrows) and the relatively few inflammatory cells. No parasites are seen in the foetal capillaries (c). Haematoxylin and Eosin, original magnification x 400.

Figure 2b: Section of placenta heavily infected with malaria showing abundant fibrinoid necrosis mainly affecting complete villi which also have pigment (arrow). Haematoxylin and Eosin, original magnification x 100.

Figure 3: Section of placenta moderately infected with malaria. Note the lymphocytic cell infiltrate (arrows). Haematoxylin and Eosin. Original magnification x 200.

Figure 4a: Section of placenta moderately infected with malaria, immunohistochemically stained with monoclonal mouse antihuman leukocyte common antigen (CD45), a pan-leukocyte marker . Note that most of the leukocytes have a brown dense reaction product in the cytoplasm and cell membrane (arrows). Original magnification x 400.

Figure 4b: Section of placenta heavily infected with malaria, immunohistochemically stained with monoclonal mouse anti-human leukocyte common antigen (CD45) as for 4a above. Note that relatively fewer leukocytes show reaction product (arrows) compared to 4a. Original magnification x 400.

Figure 5a: Section of placenta moderately infected with malaria immunohistochemically stained with mouse monoclonal antibody F8-11-13 (CD45RA), a marker for B-lymphocytes. Many of the lymphocytes show positive staining reaction product in the cytoplasm and cell membrane (arrow). Original magnification x 400.

Figure 5b: Section of placenta heavily infected with malaria immunohistochemically stained with mouse monoclonal antibody F8-11-13 (CD45RA) a marker for B lymphocytes. Note that only a few lymphocytes are positively stained, the reaction product is less dense, but its distribution is similar to 4a (arrows). Original magnification x 400.

Figure 6a: Section of placenta moderately infected with malaria, immunohistochemically stained with monoclonal mouse antihuman T cell antibody UHCLI (CD45RO). Few lymphocytes are positively stained, the reaction product is not dense and is seen mainly in the cytoplasm and cell membrane (arrows). Original magnification x 400.

Figure 6b: Section of placenta heavily infected with malaria, immunohistochemically stained with monoclonal mouse antihuman T cell antibody UHCLI (CD45RO). As in the B-cell reaction only a few lymphocytes show a weak cytoplasmic and membrane reaction product(arrows).
Original magnification x 400.

Figure 7a-b: Immunohistochemically stained sections of placenta with monoclonal mouse antihuman collagen IV:-

a) Placenta section with few malaria parasites. Note the linear mostly continuous brown reaction product along the foetal basement membrane in the villi (arrows). The trophoblastic basement membrane is unstained. Original magnification x 400.

(b) Heavily infected placenta showing reaction product that is weak, non-linear along the foetal blood vessel basement membrane which seems to be thickened (arrows). The trophoblastic basement membrane also shows a reaction product (arrow heads). Original magnification x 400.

Figure 8. Periodic Acid and Silver Methamine stained sections of placenta in :-
(a) Placenta section with no malaria parasites. Note the linear mostly continuous dark stained silver deposits on the trophoblastic and foetal basement membrane of the villi (arrows). Original magnification x 400.

(b) Placenta section heavily infected showing dark silver deposits along the foetal blood vessel and trophoblastic basement membrane which seems to be thickened (arrows). Original magnification X 400.

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