A Preliminary Study of Insulin-like Growth Factor 1 Receptor (IGF-1R) in Placental Malaria

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Abstract

Placental malaria continues to be a major health problem, causing low birthweight and maternal anemia. This study quantified the histopathology of placental malaria by staining sections with hematoxylin & eosin (H&E) and investigating the expression of insulin-like growth factor 1 receptor (IGF-1R). Through immunohistochemical staining of one control and two placental malaria cases, and by examining histological slides, placental malaria parasitemia at delivery was found to be 5% and 15%, respectively. Malaria pigment deposition was remarkable. Inflammatory cell infiltration presented in one case only. IGF-1R staining in the control showed strong expression, while placental malaria presented weak expression. The result of this preliminary study shows a need for further investigations with more cases, to confirm that insulin-like growth factor (IGF) axis disturbance may represent one mechanism by which placental malaria could lead to restricted fetal growth.

Keywords: malaria, placenta, histopathology, IGF-1R

Introduction

Plasmodium falciparum malaria affects up to 250 million people per year, and causes over 800,000 deaths annually [1]. Severe malaria is a multi-system disease with varied manifestations, including cerebral malaria, severe anemia, lactic acidosis, hypoglycemia, renal failure, and pulmonary edema [2]. In addition, severe malaria is associated with morbidity and mortality among pregnant women, affecting their fetuses and subsequently their neonates [3]. In placental malaria cases, parasitized red blood cells (PRBCs) sequester to chondroitin sulfate A expressed on trophoblast [4]. Sequestration of PRBCs results in a maternal inflammatory response that can be harmful to both mother and fetus [3,5]. During P. falciparum infection, three characteristic histological features observed in the placenta include: localization of PRBCs, inflammatory cells and deposition of hemozoin (malaria pigment), which have been suggested to represent three distinct biological processes with different temporal courses [6]. Hemozoin is seen in the intervillous space, in perivillous fibrin and in the cytoplasm of maternal macrophages [7,8], and can persist for months after heavy infection during gestation [9,10]. This study examined the histopathological features of malaria in patients, using stored placental specimens...
from the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University. A recent study has indicated that disturbance in the insulin-like growth factor (IGF) axis represents one mechanism by which placental malaria could result in restricted fetal growth [11]. Therefore, we performed a preliminary immunohistochemical investigation of IGF-1R expression in placental malaria cases.

Materials and methods
Stored placenta malaria was used from the Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University. Placental malaria specimens were collected from two patients admitted to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand. Normal control placenta was delivered with no evidence of malaria infection or any other illness complicating pregnancy or delivery. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TMEC 12-027).

Specimen preparation
Placental specimens were stored in 10% neutral buffered formalin, then processed using standard histological techniques to produce paraffin-embedded blocks. These were then cut into 4 µm histological sections on a rotary microtome, and the slides were stained with hematoxylin and eosin (H&E), or cut onto coated Supafrost slides for immunohistochemical staining.

Histopathological study
All placenta slides were examined by light microscopy. Histopathological quantitative analysis included parasitized red blood cells (PRBC) load, hemozoin (malaria pigment) load in fibrin clot and the degree of inflammatory infiltration. Other pathological features were also recorded.

Immunohistochemical study
Placenta specimens were studied for the expression of insulin-like growth factor-1 receptor (IGF-1R) β subunit. Placenta sections were stained for IGF-1R expression using an immunohistochemistry technique modified from a previous study [12]. In brief, placenta sections were deparaffinized, and rehydrated through graded ethanol. Antigen retrieval was performed by microwaving in Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 9.0). Slides were cooled in tap water, blocked with 3% peroxidase in methanol for 10 minutes at room temperature (RT), and washed in Tris-buffered saline (TBS) with 0.2% Tween-20 (TBST). After blocking with TBS containing 20% fetal calf serum (FCS) for 20 minutes at RT, slides were incubated overnight at 4°C with anti-IGF-1Rβ mouse monoclonal antibody (EMD Millipore Corporation, Massachusetts, USA) diluted 1:100 with 1% FCS in TBS. Sections were exposed to a peroxidase-conjugated polymer which carries antibodies to rabbit and mouse immunoglobulin (ChemMate, DAKO, Denmark and EnVision/HRP, Rabbit/Mouse, ENV, Denmark) for 40 minutes. After rinsing with TBS, the sections were exposed for 7 minutes to DAB+chromogen (ChemMate, DAKO, Denmark). The slides were rinsed in water and counterstained with hematoxylin. Slides were then washed in tap water, dehydrated in graded ethanol, mounted in Depex reagent and cover slipped. The results of immunohistochemical staining were examined microscopically to determine the cellular sites of IGF-1R expression.

Results
Histological findings
Through placental histological examination, the percentage of placental malaria at delivery in case 1 and case 2 was 5% and 15% respectively. PRBC in placental malaria cases were observed in maternal erythrocytes in the intervillous space (Fig 1A), but not in fetal erythrocytes within villi which would indicate congenital infection. Cytoadherence of PRBC to syncytiotrophoblast epithelial cells was found on the surface of villi (Fig 1B). The inflammatory infiltrate was composed predominantly of monocytes. Malaria pigment was most commonly found in intervillous areas of fibrin clot, monocytes in the intervillous space,
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and villous epithelial cells, and malaria pigment deposits in the perivillous fibrin of the placenta. Other pathological features were also recognized including intervillous fibrin clot.

Immunohistochemistry for IGF-1R

IGF-1R staining was expressed in the syncytiotrophoblasts, cytotrophoblasts and endothelial cells of placental specimens in all cases.

Fig 1  (A) Parasitized red blood cells (PRBC) (arrows) present in the intervillous space (H&E, ×1,000); (B) Cytoadherence (arrows) of PRBCs in placenta (H&E, ×1,000).

Fig 2  Immunohistochemical staining for IGF-1R in the placenta (×400). (A) Negative control showing negative staining for IGF-1R; (B) Normal control showing strong positive staining for IGF-1R in a granular cytoplasmic pattern; (C) and (D) Placental malaria showing weak staining of IGF-1R (arrows).
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The staining was localized mainly in the cytoplasm or cell membrane, but occasionally nuclear staining was also seen (Fig 2). IGF-1R expression was strongest in normal control patient, as had been expected and also mentioned in previous reports that this antigen was constitutively expressed on villi in normal term placenta. Placental malaria seemed to show lower levels of IGF-1R expression than those in normal control.

Discussion

Several pathological studies have been conducted in which placental biopsies were examined from malaria patients after delivery. The findings show the collection of PRBC in the maternal villous blood space, host inflammation, and the deposition of malaria pigment in fibrin clots. Other pathological features were also recognized, including intervillous fibrin clot, hemorrhage, changes to villous architecture, and syncytial knots. As a result of these studies and their important findings, a number of different histological grading schemes have been proposed to measure pathology in the placenta. These include the schemes of Bulmer et al [13], and more recently Muehlenbachs et al [14]. Our study of two cases of acute placental malaria also showed PRBC and malaria pigment in both cases, but little acute inflammation.

Cytoadherence between PRBC and syncytiotrophoblast was found in our study. There is an indication that adhesion of PRBC to syncytiotrophoblastic cells initiates signaling in these cells, evidenced by tyrosine phosphorylation [15]. In addition, both malaria pigment [16] and host leukocytes cause the release of a number of cytokines and chemokines in the placenta, which can attract host monocytes [17] and cause autoinduction of release of both chemokines and angiogenic factors from both host leukocytes and syncytiotrophoblastic cells [18-20].

Although there have been fewer studies on the role of growth factors on villous development, there has been interest in the effects of various inflammatory cytokines and mediators on villous development and function in placental malaria. There were some studies conducted on IGF-1R, a receptor for IGF-1 and IGF-2, which has been implicated in support of normal placental growth and development [21,22]. Subsequently in 2011, Umbers et al examined soluble factors and growth factors changing in the placenta and confirmed that mRNA for IGF-1 decreased significantly. Serum or cord blood levels of IGF-1 were also significantly reduced in maternal malaria [23]. The possibility exists that reduction in the IGF signaling axis inhibits the ability of the placenta to grow and function properly.

Immunohistochemical expression of IGF-1R protein in placenta during malaria infection was performed in this study. The expression of IGF-1R was significantly reduced in active malaria infection.

Table 1 Summary of histological scoring and IGF-1R expression.

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<thead>
<tr>
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<th>Normal control</th>
<th>Placental malaria</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
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<tbody>
<tr>
<td>Parasitemia (%)</td>
<td>0</td>
<td>5</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Malaria pigment score</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IGF-1R expression</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
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Note: Malaria pigment score: score 0 (0%), score 1 (< 10%), score 2 (10-40%) and score 3 (> 40% of fields positive). The inflammation score: minimal inflammation (Score 1), inflammation present (Score 2) and massive intervillositis (Score 3).
cases when compared to normal control. However, the decreased expression of IGF-1R seen here would be in keeping with a general suppression of the IGF axis during malaria, as shown in previous studies of IGF levels in maternal malaria [11,23].

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References


