

Change of serum transferrin receptor due to malarial infection, an experiment in *Plasmodium gallinaceum* infected chicken model

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Abstract

Background & objectives: The serum transferrin receptor (sTfR) concentration in an individual reflects the extent of erythropoietic activity and is considered as an useful marker of iron deficiency independent of concurrent inflammation or infection. However, data on the impact of malaria on this parameter are ambiguous.

Methods: Here we performed an animal experiment to study the chronological change of serum transferrin receptor due to infection of *Plasmodium gallinaceum*. In this pilot study, we performed control experimental infection of *P. gallinaceum* to four newborn chicken from the same batch. We collected the venous blood samples from all chicken on Day 7 and 14. All samples were analysed for sTfR level by the immunoturbidimetric assay.

Results: The average level of sTfR level of the control chicken was 1.24 ± 1.58 mg/L (range 0.18 to 3.52 mg/L). The average level of sTfR level of the experimental chicken on Day 7 was 5.42 ± 2.19 mg/L (range 3.22 to 13.94 mg/L).

Conclusion: Although the trend of increase was observed but no significance was observed ($p > 0.05$). The results from this pilot study can be a good basic data for the further study in this area.

Key words Chick – immunofluorimetric assay – malaria – *P. gallinaceum* – serum transferrin

Introduction

Transferrin receptor (TfR) is a glycoprotein found in animals, which mediates the entry of ferric transferrin from the extracellular compartment into the cells¹. Up-regulation of the expression of cellular TfR occurs as a result of an inadequate tissue supply of iron or increased cellular demand for iron, therefore, elevation of soluble form of TfR (sTfR) can be detected in any disease causing alteration of iron metabolism especially for the anaemia and haemolysis.

In a clinical setting, sTfR measurements have been widely used and offer an attractive amendment to the repertoire of indices of iron status. The sTfR concentration has also been shown to be a more sensitive and less variable index of iron status than the more conventional serum iron, transferrin, and total iron-binding capacity^{1–3}.

Presently, the measurement of sTfR has become a widely used tool in assessing iron status, but its use has mainly been restricted to research laboratories.

Presently, the changes of sTfR level are studied in only a few diseases, especially for the iron deficiency anaemia^{1,4-6} and thalassemia⁷. Here, we report our experience from a preliminary study on the change of sTfR level in malaria using the animal model.

Material & Methods

Animal experiment: We performed an animal experiment to study the chronological change of sTfR due to control infection of *Plasmodium gallinaceum*. In this pilot study, control experimental infection of *P. gallinaceum* to four newborn chicken (Day 0) from the same batch at the Veterinarian Parasitology Laboratory, Chulalongkorn University, Bangkok, Thailand was performed. All chicken were injected with 5×10^4 *P. gallinaceum* subcutaneously. A new Thai strain obtained from Nithiuthai S, Chulalongkorn University, Bangkok, Thailand⁸ was used in this study. All chicken in this study were fed with the same food and put in the same environmental conditions. All chicken were confirmed for infection on the first blood sampling on Day 7. All chicken did not get any antimalarial drug till the experiment was completed.

Study of change of the sTfR due to malarial infection: We performed the experimental infection to four chicken to be the animal model for the study. Since we could not get the blood sample from the newborn <7 days chicken because the chicken would die if we do so, therefore, we collected the sample after seven days (average percentage of parasitaemia = $3 \pm 3.1\%$, Hb = 7.8 ± 1 g/dl, PCV = $23.8 \pm 6.8\%$) and 14 days (average percentage of parasitaemia = $3.4 \pm 3.5\%$, Hb = 7.4 ± 1.3 g/dl, PCV = $23.4 \pm 5.6\%$) to study the chronological trend of the sTfR change. For each sample collection, 1 ml of blood sample was collected from each chick. In addition, we set the other four chicken from the same batch, same feeding and environment condition, to be the control non-infected group. Control sera from the four normal chicken were also collected on Day 14 for comparison to the experimental group.

Determination for sTfR: All samples were analysed for sTfR level by the immunoturbidimetric assay. The studied assay is a particle enhanced immunoturbidimetric assay of soluble transferrin receptor, IDEAS[®] sTfR-IT (Orion Diagnostica; Espoo, Finland). This assay has been evaluated for its analytical performance in our previous study and found to be acceptable⁹. Briefly, the assay is based on the detection of an immunoreaction between sTfR and sTfR-specific antibodies in liquid phase. The immunoreaction is enhanced by particles coated with sTfR-antibodies. Measurement is performed by photometry range at 540 to 690 nm. The amount of immunoprecipitate is proportional to the sTfR concentration in the sample. In this study, all tests were performed using the automated clinical chemistry analyser, Hitachi 911 (Roche-Boehringer Mannheim). All analyses were performed according to the manufacturer's instructions. The used assay was confirmed for its good diagnostic property with our previous study⁹.

Statistical analysis: Average sTfR levels (mean \pm S.D.) of each group of subjects were calculated. Non-parametric statistical analysis was used for the determination of statistical difference between averages using SPSS 10 for Windows.

Results

The sTfR level of the experimental chicken and the trend of change: The average level of sTfR level of the experimental chicken on Day 7 was 5.42 ± 2.19 mg/L (range 3.22 to 13.94 mg/L). The average level of sTfR level of the experimental chicken on Day 14 was 7.76 ± 4.56 mg/L (range 2.64 – 7.7 mg/L). Although the trend of increase was observed but no significance difference was observed ($p > 0.05$).

The sTfR level of the control non-infected chicken: The average level of sTfR level of the control chicken (Day 14) was 1.24 ± 1.58 mg/L (range 0.18 to 3.52 mg/L). This level was significantly less than those of the experimental chicken (Day 14) ($p < 0.05$).

Discussion

Cellular iron uptake in vertebrate animal is mediated by transferrin receptors (TfR). A soluble form of TfR (sTfR) detected in serum is closely related to erythroid TfR turnover. Increased erythropoietic activity causes TfR synthesis to be upregulated and thereby increase the soluble transferrin receptor (sTfR) level. Determination of sTfR concentration can reflect cellular iron demands and the erythroid proliferation rate⁵. Therefore, measurement of sTfR has been introduced as a powerful tool for monitoring of erythropoiesis in a variety of clinical situations^{1,4-6}.

The sTfR concentration in an individual reflects the extent of erythropoietic activity and is considered a useful marker of iron deficiency^{1,3}. Iron deficiency is highly prevalent in most developing countries, however, its detection is often obscured by infections and inflammatory disorders which are common in the same populations such as iron deficiency anaemia (IDA) as iron deficiency with concurrent anaemia. Nevertheless, ineffective erythropoiesis was described in studies of the bone marrow of patients with anaemia as well as malaria¹⁰. These studies found abnormalities in developing erythroblasts and evidence of increased phagocytosis of erythroblasts at various stages of degradation. The sTfR is, therefore, questionable that it can be a single indicator of iron deficiency since it might be affected by malaria or inflammation.

There are several studies to answer this question. However, data on the impact of malaria on this parameter are ambiguous. Stoltzfus *et al*¹¹ found that sTfR concentrations increased in children with asymptomatic malaria. Several other studies support the finding of increased sTfR concentration in persons with asymptomatic malaria. Mockenhaupt *et al*¹² found increased sTfR concentrations in persons with asymptomatic and mildly symptomatic falciparum malaria. However, Huddle *et al*¹³ found that malaria was not associated with sTfR concentrations.

In addition, a decrease in sTfR concentrations was reported by Williams *et al*¹⁴.

Since most studies were the descriptive, describing the finding from the observation in the infected comparing to non-infected cases, the conclusion could not be drawn. Nevertheless, most of interferences on those observations, especially for the nutritional status on the sTfR level could be expected. Here we performed an animal experiment to study the chronological change of sTfR due to control infection of *P. gallinaceum*. The interference from the nutritional status on the sTfR level can be controlled. According to our study, we detect the trend that sTfR level increases according to the infection period although it is not significant. The average among the infected subjects was higher than that of the non-infected ones. Similar findings were reported by Mockenhaupt *et al*¹² and Stoltzfus *et al*¹¹ as well.

Although this study is only a small pilot study but it is the first report of experimental study of the effect of malarial infection *in vivo*. Our results support those observations on the increase of sTfR level in malaria. The increase in sTfR level in cases of infected chicken than the normal ones might indicate that parasite needs *de novo* transferrin for their growth or host cell needs to recoup with the loss of iron (which might be confirmed by the observation of anaemia in the infected chicken). This point has to be studied in further works. Since a number of tropical diseases including iron deficiency anaemia and thalassemia can present the increase in sTfR as well, the interpretation of sTfR level as the single parameter for assessment of iron status must be careful. The results from this pilot study can be a good basic data for the further study in this area.

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