MAJOR ARTICLE

Soluble Tumor Necrosis Factor-α Receptor 2 in Urine Is a Potential Biomarker for Noninvasive Diagnosis of Malaria During Pregnancy

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Background. During pregnancy, the placenta is inaccessible for diagnosis of placental malaria (PM), but soluble tumor necrosis factor-α receptors (sTNFR) are elevated in the plasma of women with PM.

Methods. In this study, sTNFR-1 and sTNFR-2 were quantified in urine of pregnant and nonpregnant Cameroonian women who were positive or negative for malaria by blood-smear microscopy.

Results. We found that levels of both sTNFR in urine were higher in pregnant compared with nonpregnant women, but malaria-positive pregnant women excreted substantially more sTNFR-1 ($P = .005$) and sTNFR-2 ($P < .001$) than malaria-negative pregnant women. The amount of sTNFR-1($r_s = 0.784$, $P < .001$) and sTNFR-2 ($r_s = 0.816$, $P < .001$) in urine correlated with parasitemia, even in afebrile pregnant women. Urine sTNFR-2 predicted maternal malaria with an area under curve of 0.892 (95% confidence interval, .787–.898). At cutoff concentrations of 9.8 ng and 13.6 ng of sTNFR-2 per mL urine, the sensitivity/specificity were 82.6%/87.0% and 78.3%/95.7%, respectively.

Conclusions. The sTNFR-2 in noninvasive urine samples may be useful for diagnosis of malaria during pregnancy.

Keywords. biomarker; malaria; pregnancy; TNF-α receptors; urine.

Infection by the malaria parasite *Plasmodium falciparum* during pregnancy is associated with significant maternal and neonatal morbidity [1]. *Plasmodium falciparum*-infected erythrocytes (IE) adhere to placental villi [2, 3], which leads to placental inflammation and poor pregnancy outcomes such as maternal anemia, preterm deliveries, and low birth weight (LBW) babies [4–7]. Prompt treatment is important in reducing this burden, but diagnosing malaria during pregnancy can be difficult. The diagnosis of placental malaria (PM) is confirmed by detection of malaria parasites in placental impression smears and histosections [8, 9]. However, the placenta is accessible only after delivery, ie, when the fetus has already suffered malaria-associated complications. During pregnancy, peripheral blood smears are often used for microscopic diagnosis of malaria. Because IE sequester in the placenta, 20%–50% of women with PM may test negative on peripheral smears [8, 10]. Furthermore,

identification of IE, parasite deoxyribonucleic acid, and parasite proteins in peripheral blood by microscopy, polymerase chain reaction, and rapid diagnostic tests (RDT), respectively, are not indicative of placental inflammation, which is a major factor in the pathogenesis of malaria-associated adverse obstetrical outcomes [5, 11, 12]. Collection of blood is also associated with pain and risk of infection at sites of venipuncture. Therefore, if PM-associated inflammatory biomarkers could be detected in noninvasive biological samples, such as urine, and are accurate in predicting PM, significant progress would be made towards early diagnosis and treatment of PM.

Soluble receptors of tumor necrosis factor (TNF)-α are among the promising candidate biomarkers of PM [13, 14]. Tumor necrosis factor-α is a 150-kDa polypeptide produced mainly by activated macrophages and is involved in a variety of bioactivities, including cytotoxicity and inflammation [15–18]. Tumor necrosis factor-α is necessary for malaria parasite clearance, but excessive TNF-α levels can be detrimental [19]. Placental malaria is associated with an increase in TNF-α levels in peripheral and placental intervillous space (IVS) blood [20]. High plasma levels of TNF-α during first pregnancies have been linked with severe anemia and LBW [21, 22]. Tumor necrosis factor-α functions by binding to 2 membrane receptors: a 55- to 60-kDa TNFR-1 and a 75- to 80-kDa TNFR-2, on the surface of target cells [23, 24]. Both receptors are shed by proteolytic cleavage into circulation as soluble TNF-α receptors-1 (sTNFR-1) and 2 (sTNFR-2). At low concentrations, sTNFR stabilize TNF-α and enhance its activities [25], whereas at high concentrations

they antagonize TNF-α binding to membrane receptors [26]. Plasma levels of sTNFR-1 and sTNFR-2 correlate with plasma TNF-α concentrations, parasitemia and malaria severity in children [27], and nonimmune adults [28, 29], indicating an increased release of sTNFR in response to TNF-α secretion during malaria.

The placenta is a source of sTNFR, and the detection of sTNFR in urine might be an alternative method for diagnosing PM pathology. Expression of sTNFR-1 messenger ribonucleic acid (mRNA) in placental tissue [30] and levels of sTNFR proteins in maternal blood [31] and urine [32] increase during the second half of normal pregnancy. Increased release of sTNFR-1 and sTNFR-2 into peripheral plasma has been reported in pregnant primates inoculated with *Plasmodium coatneyi* [33] and in pregnant women after natural *P falciparum* infections [13]. In addition, plasma sTNFR-2 levels correlate with maternal *P falciparum* parasitemia and are also elevated in pregnant women with submicroscopic parasitemia [13], postulating sTNFR-2 in peripheral plasma as a biomarker of PM. However, an observation that sTNFR concentrations during normal pregnancies are higher in urine than in matched plasma samples [34] suggests that a urine-based detection of sTNFR would be more sensitive than a blood-based test. In the present study, urine samples from a group of Cameroonian women were assayed for sTNFR-1 and sTNFR-2 to determine whether malaria in pregnancy influences the excretion of these receptors into urine and to assess the accuracy of quantifying sTNFR-1 and sTNFR-2 in urine for diagnosis of malaria in pregnant women.

MATERIALS AND METHODS

Study Population and Sample Collection

Pregnant and nonpregnant women were recruited between August 2009 and January 2010 at 3 health facilities in and around Yaoundé Cameroon where malaria is holoendemic. Pregnant women attending antenatal clinics at the Yaoundé Central Hospital, Biyem-Assi District Hospital, and Ntouessong Health Center were screened for malaria using RDT (CareStart Rapid one-step malaria pLDH/HRP-2; Access Bio Inc.). Approximately 20 mL of clean-catch midstream urine and 1 mL venous blood were collected from RDT-positive and gestational age-matched RDT-negative pregnant women. Matching was done because it has previously been reported that levels of sTNFR in urine increase with the duration of normal pregnancy [32, 34]. Nonpregnant women were recruited at outpatient units and from communities around the health facilities. Dipstick urinalysis (Combina 11 S; Human, Germany) was performed at the recruitment sites to detect nitrate-reducing bacteria, and the axillary temperatures were measured. Women with known human immunodeficiency virus (HIV) infection, diabetes, rheumatoid arthritis, and other chronic diseases were excluded, as well as pre-eclamptic and parturient women. To avoid blood contamination of urine samples, all women with ongoing vaginal bleeding of any etiology were also excluded. All participants signed an informed consent form. All RDT-positive women were directed to their attending physicians for malaria treatment. The study was approved by the Institutional Review Board of the University of Hawaii, United States and the National Ethics Committee for Research on Human Subjects of the Ministry of Public Health, Cameroon.

Determination of Packed Cell Volume, White Blood Cell Counts, and *Plasmodium falciparum* Parasitemia

Packed cell volume (PCV) was measured by assessing the proportion of packed cells to total blood volume after centrifugation of whole blood in capillary tubes. White blood cell (WBC) counts were determined using an automated hematology analyzer (URIT-3300 Hematoanalyzer, Diamond Diagnostics, USA). Thick and thin blood smears were air dried, stained with Giemsa, and examined by 2 experienced microscopists. The number of parasites/μL blood was determined by multiplying the number of parasites/200 WBC by the number of WBC/μL blood. Microscopy and field RDT results were used to categorize the participants into malaria-positive or malaria-negative groups.

Urine Soluble Tumor Necrosis Factor-α Receptor 1 and 2 Enzyme-Linked Immunosorbent Assay

All urine samples were aliquoted and stored on the day of collection at −20°C until analyzed. The sTNFR-1 and sTNFR-2 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Hycult Biotechnology b.v., The Netherlands) following manufacturer's instructions. In brief, 100 μL 1:50 diluted urine samples and reconstituted control standards were added in duplicates into microplate wells that were precoated with anti-TNFR-1 or anti-TNFR-2 antibodies. After 2 hours, plates were washed and a 100-μL biotin-coupled anti-TNFR antibody was added into each well. The plates were incubated for 1 hour, washed, and 100 μL/well of diluted streptavidin peroxidase was added. After 1 hour, 100 μL/well of trimethyl benzidine substrate was added. The reaction was stopped after 25 minutes by adding 100 μL of 2 M citric acid. All washes were conducted 3 times, and all incubations were at room temperature. The optical densities (ODs) at 450 nm were measured by spectroscopy. Duplicate ODs were averaged, and net ODs were calculated by subtracting the blank average OD. The concentrations of sTNFR-1 and sTNFR-2 in nanograms per milliliter were calculated by extrapolation from standard curves and multiplying by the dilution factor.

For confirmation, all urine samples were retested for sTNFR-1 and sTNFR-2 using bead-based Luminex assays. The assays were performed using 50 μL 1:50 diluted urine and according to guidelines from the manufacturer (TNF-RI and TNF-RII Human Kits for Luminex Platform; Invitrogen Corporation). For comparison of sTNFR diagnostic accuracy with that of a malaria parasite-specific protein, histidine-rich protein 2 (HRP-2) of *P falciparum* was also measured by ELISA as previously described [35] using 100 μL undiluted urine.

Statistical Analyses

Data were analyzed using GraphPad Prism 5.03 (GraphPad Software, Inc). Medians and interquartile ranges (IQRs) or means and standard deviations were used to describe central tendency and variation for variables with skewed distribution or normal distribution, respectively. Fisher's exact test was used to compare proportions. Nonparametric tests were used to compare urine sTNFR levels across multiple groups (Kruskal–Wallis test, with Dunn's correction) and between groups (Mann–Whitney U test). Correlation analyses were done using the Spearman rank test and partial correlation coefficients. Finally, the diagnostic accuracy of each soluble receptor in urine was assessed by receiver operating characteristic (ROC) curve analysis.

RESULTS

Participant Characteristics

A total of 66 women were included in the study: 23 were pregnant and slide positive for malaria, 23 were malaria-negative pregnant women, and 20 were nonpregnant women (10 positive and 10 negative). Table 1 summarizes the characteristics of the study groups. As expected, there was no difference in gestational age ($P = .493$) between malaria-positive and malaria-negative pregnant women because they were matched for gestational age during recruitment. Malaria-negative and malaria-positive pregnant women were also of similar ages ($P = .538$) and gravidity ($P = 1$). The majority (85%) of women were afebrile, with only 10 (6 pregnant and 4 nonpregnant) of 66 women having temperatures >37.5°C. All febrile women were malaria positive, and parasitemia was higher in febrile compared with nonfebrile malaria-positive participants ($P = .003$). White blood cell counts were similar between malaria-positive and malaria-negative women in both pregnant ($P = .908$) and nonpregnant ($P = .232$) groups. None of the participants had clinically overt

foci of microbial infection, and urine nitrites, which are indicative of nitrate-reducing bacteriuria, were positive in only 3 pregnant women who were also afebrile and *P falciparum* negative. Overall, the results suggest, but are not conclusive of, a low likelihood of nonmalarial infections or coinfections in the study population.

Urine Levels of Soluble Tumor Necrosis Factor-α Receptors 1 and 2 Are Influenced by Malaria and Pregnancy

Among all malaria-negative participants, pregnant women had higher levels of urine sTNFR-1 ($P < .001$) and sTNFR-2 ($P = .005$) than nonpregnant women (Figure 1). In addition, malaria-positive pregnant women had significantly higher levels of sTNFR-1 ($P = .005$) and sTNFR-2 ($P < .001$) in urine than pregnant women who were malaria negative (Figure 1A and 1B). The median sTNFR-2 concentrations in the urine of nonpregnant malaria-negative, pregnant malaria-negative, and pregnant malaria-positive women were 1.5 ng/mL, 6.0 ng/mL, and 49.0 ng/mL, respectively, representing a 4-fold pregnancy-related increase and an 8-fold malaria-related increase. Nonpregnant malaria-positive women had significantly more urine sTNFR-1 ($P = .009$) and sTNFR-2 ($P < .001$) than nonpregnant malaria-negative women but lower levels of both receptors than pregnant positive women, albeit not significant. Collectively, the results show that normal pregnancy is associated with increased sTNFR excretion in urine as previously reported [32, 34] and further demonstrate a more substantial malaria-associated increase in urine sTNFR excretion especially during pregnancy.

Next, correlation between sTNFR in urine and *P falciparum* parasitemia was assessed. Urine levels of each soluble receptor increased with increasing peripheral parasitemia (Figure 2A and 2B), and strong positive correlations between parasitemia and urine concentrations of sTNFR-1 (Spearman $r_s = 0.784$, $P < .001$) and sTNFR-2 ($r_s = 0.816$, $P < .001$) were found.

Table 1. Description of Study Participants[a]

Characteristics	Pregnant Women			Nonpregnant Women		
	Malaria Negative	Malaria Positive	P Value	Malaria Negative	Malaria Positive	P Value
Number	23	23		10	10	
Age in years[b]	25.1 ± 5.6	24.0 ± 5.2	.538	26.4 ± 4.7	24.7 ± 6.1	.499
Gestational age in weeks[b]	26.9 ± 9.2	25.1 ± 9.6	.493	N/A	N/A	
Primigravid[c]	8 (34.8%)	9 (39.1%)	1.0[b]	N/A	N/A	
Temperature >37.5°C[c]	0 (0%)	6 (26.1%)	**.022**[d]	0 (0%)	4 (40%)	.087[d]
WBC count, cells/μL blood[b]	6741 ± 1010	6798 ± 2065	.908	5133 ± 1109	6280 ± 1846	.232
PCV in %[b]	35 ± 3.1	32 ± 3.1	**.002**	37 ± 5.9	37 ± 4.3	.925
Parasitemia, parasites/μL blood[e]	N/A	6290 [768–23 672]		N/A	8849 [2049–48 240]	
Presence of UTI[c]	3 (13%)	0 (0%)	.233[d]	0 (0%)	0 (0%)	N/A

Abbreviations: N/A, not applicable; PCV, packed cell volume; UTI, urinary tract infection; WBC, white blood cells.

[a] Malaria-positive or -negative status was determined by blood-smear microscopy.

[b] Mean ± standard deviation.

[c] Number (percent).

[d] Fisher's exact test was used to calculate P values. All other P values were calculated using Student t test. Bold text indicates significant P values.
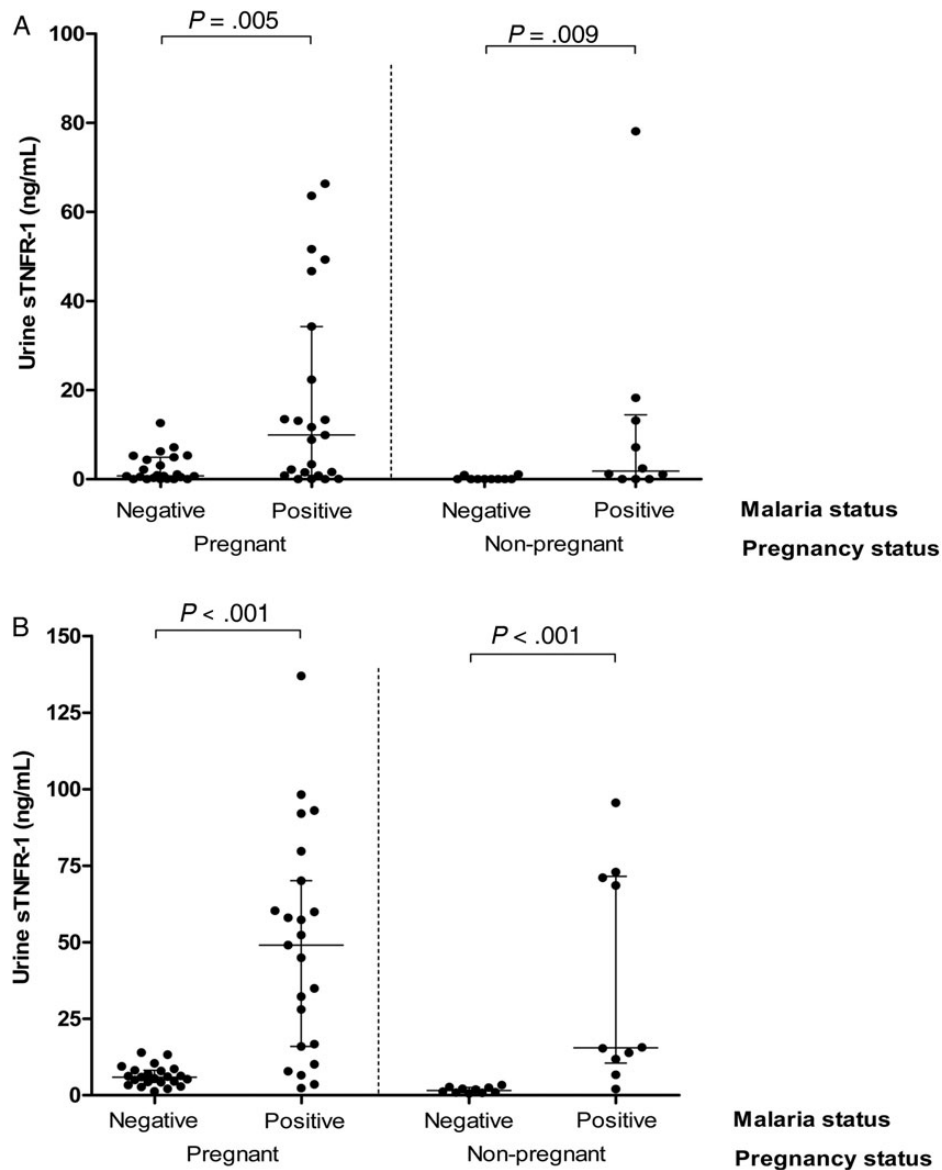
[e] Median [25th–75th percentile].

Figure 1. Concentrations of soluble tumor necrosis factor receptor-α (sTNFR)-1 (A) and sTNFR-2 (B) in urine of pregnant and nonpregnant women who were slide positive or negative for *Plasmodium falciparum*. Soluble TNF-α receptor levels were measured in the urine of 66 women: 23 were pregnant and negative for *P falciparum*; 23 were pregnant but malaria negative; 10 were nonpregnant malaria positive; and 10 were nonpregnant malaria negative. Each dot indicates the concentration for 1 woman. Horizontal lines represent 25th, 50th, and 75th percentiles. Heights of vertical lines represent interquartile ranges. The *P* values were obtained from Mann-Whitney *U* tests.

Other variables such as maternal age, gestational age, gravidity, body temperature, and PCV were also analyzed for association with urine sTNFR. Only body temperature (sTNFR-1: r_s = 0.362, P = .002 and sTNFR-2: r_s = 0.420, P < .001) and PCV (sTNFR-2: r_s = −0.341, P = .005) were significantly but moderately associated with urine levels of sTNFR. Because all participants with fever were slide positive for *P falciparum*, the temperature effect on urine sTNFR would largely be an indirect effect of malaria. The association between parasitemia and sTNFR concentrations in urine remained significant even when the effects of body temperature and anemia were

controlled for using partial correlation coefficients (partial r = 0.556, P = .001 for sTNFR-1 and partial r = 0.634, P < .001 for sTNFR-2). Taken together, these results show that women infected with the malaria parasite during pregnancy release high levels of sTNFR into their urine and that these receptor levels correlate with parasitemia even in the absence of fever.

Performance of Urine Soluble Tumor Necrosis Factor-α Receptors 1 and 2 in Diagnosing Malaria in Pregnancy

Because urine sTNFR-1 and sTNFR-2 were significantly elevated in malaria-positive pregnant women, ROC curve analysis was used to investigate the performance of the 2 receptors as diagnostic
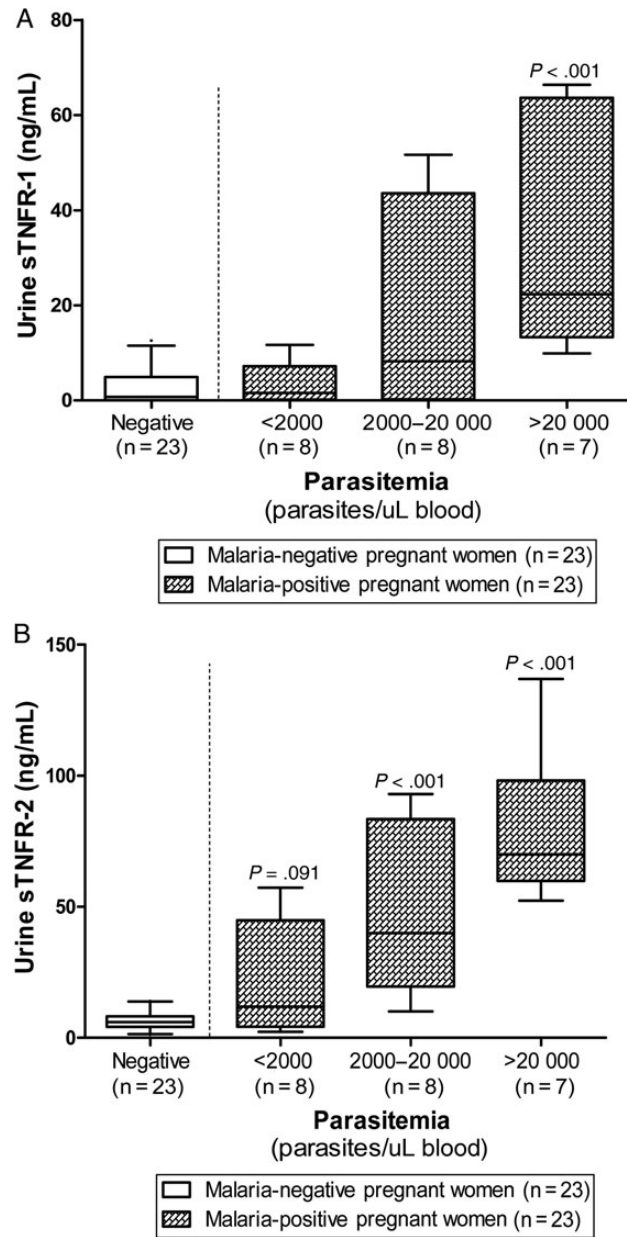
Figure 2. Association between soluble tumor necrosis factor-α receptor (sTNFR) concentrations in urine and peripheral parasitemia. (A) sTNFR-1 and (B) sTNFR-2. Malaria-positive pregnant women were categorized into 3 groups of increasing peripheral parasitemia (<2000, 2000–20 000, and >20 000 *Plasmodium falciparum* parasites/μL blood), and their urine levels of sTNFR were compared with those of malaria-negative pregnant women. The *P* values were determined by the Mann–Whitney *U* test and indicate the level of significance of the difference in sTNFR between each positive category and the negative group. Results are represented in box-and-whisker plots where horizontal lines within boxes denote medians, heights of boxes denote interquartile ranges, and bars designate range from 5th percentile to 95th percentile. Note: pregnant women in the 3 categories of peripheral parasitemia did not differ significantly in their gestational age (*P* = .246).

urine biomarkers of malaria in pregnancy (Figure 3A and 3B). The ROC analysis was not done for nonpregnant women because of the smaller sample size. The detection of sTNFR-1 in urine had a moderate diagnostic accuracy with an area under curve (AUC) of 0.748 (95% confidence interval [CI], .598–.898) (Figure 3A). However, urine sTNFR-2 had a larger AUC of 0.892 (95% CI, .787–.998) (Figure 3B) and was significantly more accurate than sTNFR-1 (*P* = .02) in predicting malaria in pregnant women.

Using a cutoff concentration of 9.8 ng/mL, sTNFR-2 had a sensitivity and specificity of 82.6% and 87.0%, respectively. At 13.6 ng/mL, the sTNFR-2 sensitivity and specificity were 78.3% and 95.7%, respectively. In contrast, the best sTNFR-1 cutoff of 26.1 ng/mL yielded a sensitivity of only 56.5%.

The above-mentioned AUCs were calculated using sTNFR-1 and sTNFR-2 concentrations in urine measured by ELISA, which was the main assay of the study. To ascertain the diagnostic
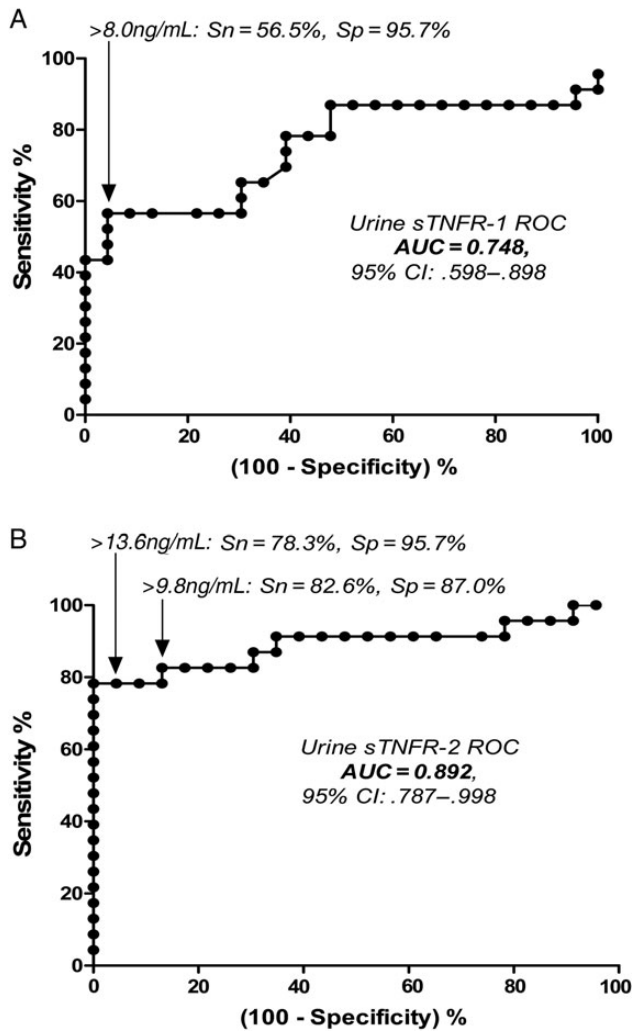
Figure 3. Performance of urine soluble tumor necrosis factor-α receptor (sTNFR) in diagnosing malaria in pregnancy. Receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of urine sTNFR-1 (A) and sTNFR-2 (B) in 46 pregnant women. The area under ROC curve (AUC) with 95% confidence interval (CI) was 0.748 (CI, .598–.898) for sTNFR-1 and 0.892 (CI, .787–.998) for sTNFR-2. Arrows point at cutoff concentrations where the best sensitivity (Sn)/ specificity (Sp) pairs were recorded for each soluble receptor.

Table 2. Reproducibility of Urine sTNFR Performance and Comparison With Urine HRP-2 Performance in Diagnosing Malaria During Pregnancy[a]

Urine Biomarker	AUC	95% CI of AUC	P Value of AUC
sTNFR-1[b]	0.741	.593–.889	**.005**
sTNFR-1[c]	0.771	.633–.909	**.002**
sTNFR-2[b]	0.892	.787–.998	**<.001**
sTNFR-2[c]	0.875	.765–.986	**<.001**
HRP-2[b]	0.547	.369–.724	.606

Abbreviations: AUC, area under receiver operating characteristic curve; CI, confidence interval; ELISA; enzyme-linked immunosorbent assay; HRP-2, histidine-rich protein-2; sTNFR, soluble tumor necrosis factor-α receptor.

[a] AUCs were used to assess the diagnostic accuracy (1) for sTNFR measured in urine by ELISA and Luminex and (2) for HRP-2 by ELISA. The reference AUC of an ideal test with 100% sensitivity and 100% sensitivity is 1, whereas that of an indiscriminate irrelevant test is 0.5. Bolded P values are significant.

[b] Detection and quantification by ELISA.

[c] Detection and quantification by Luminex.

performance of urine sTNFR, bead-based Luminex assays were also used to detect and quantify the soluble receptors in the same urine samples. For both sTNFR-1 and sTNFR-2, the AUC from Luminex-derived data were similar to AUC of from ELISA-derived data (Table 2). Lastly, the urine samples were screened by ELISA for HRP-2, but urine HRP-2 was very inaccurate as a diagnostic biomarker for malaria in pregnancy (AUC = 0.547, P = .606) (Table 2).

DISCUSSION

The identification of biomarkers of maternal malaria is crucial because malaria in pregnancy is associated with maternal and fetal morbidity, and current methods of diagnosing PM before

birth are inadequate. This study showed that maternal malaria is associated with significant excretion of TNF-α soluble receptors in urine. The sTNFR-1 and sTNFR-2 concentrations in urine were elevated as part of normal pregnancy but were a lot higher during malaria in both pregnant and nonpregnant women and highest in malaria-positive pregnant women.

The fact that normal pregnancy on its own induces the release of sTNFR and that IE sequester in the placenta suggest that much of the soluble receptors detected in the urine of pregnant women with malaria are produced by the placenta. In addition, sTNFR-1 mRNA has been detected in placental trophoblastic and mesenchymal tissue during late gestation [30]. Furthermore, purified term villous trophoblasts and BeWo cells extensively shed both receptors in vitro [36]. Besides trophoblastic tissue, monocytes that accumulate in the IVS during PM [5, 6] are important sources of sTNFR [17]. However, the malaria-associated release of sTNFR from constitutive and infiltrating cells in the placental environment may not be a direct result of interaction with IE. The sTNFR are released in reaction to inflammatory mediators secreted in response to IE sequestration. A previous study showed that BeWo cells and THP-1 cells released sTNFR in response to phorbol 12-myristate 13-acetate and not after coculture with IE only [13]. In addition, the cytokine interleukin (IL)-10, which is released in significant amounts during PM [14], is known to enhance the release of sTNFR-2 from monocytes [37, 38].

To ensure that the difference in urine sTNFR concentrations between malaria-positive and malaria-negative pregnant women was due to malaria, potential confounding factors were controlled. Levels of sTNFR-1 have been shown to increase in pre-eclampsia [39, 40] so all women with this condition were excluded from the study. All HIV-positive women and women with other chronic diseases were also excluded. Malaria-positive and malaria-negative women were also matched for gestational age because TNF-α receptor shedding is higher in more advanced pregnancy [32]. Univariate analyses did not show any

association between soluble receptor levels and maternal age or gravidity. Of note, sTNFR-1 and sTNFR-2 concentrations in urine increased with body temperature. This is partly due to a physiologic increase in protein excretion in urine during periods of fever, but it also implies that any infectious process or inflammation at the origin of the fever could have contributed to the receptor shedding. However, malaria infection must have been the major contributor, because all febrile participants were positive for malaria, and WBC counts were similar between malaria-positive and malaria-negative pregnant women.

Three women were positive for nitrate-reducing bacteriuria, but all of them were malaria negative, and their sTNFR-2 urine concentrations of 4.1 ng/mL, 6.0 ng/mL, and 6.1 ng/mL, respectively, were lower than the 25th percentile value for malaria-positive pregnant women (median = 30.3 ng/mL, IQR = 11.6–84.9 ng/mL). Thus, urinary tract infections did not significantly influence sTNFR levels in this present study. Lastly, peripheral parasitemia showed a strong positive correlation with the concentrations of sTNFR-1 and sTNFR-2 in urine. Although parasites may be absent at the periphery during PM, increasing peripheral parasitemia reflects higher placental parasite burden and inflammation [10]. Collectively, the data show that concentrations of sTNFR in urine are strongly influenced by maternal malaria.

The final objective of this study was to assess the usability of urine sTNFR as biomarkers for malaria diagnosis during pregnancy. Urine sTNFR-2 was more accurate in predicting malaria than sTNFR-1. The area under ROC curve, which is a measure of the diagnostic accuracy of a biomarker, was high for sTNFR-2 (AUC = 0.892) (Figure 3A) but only moderate for sTNFR-1 (AUC = 0.748) (Figure 3B). When the samples were rerun in a Luminex-based assay, the sTNFR-2 AUC remained significantly high (AUC = 0.875) (Table 2), indicating that the results were reproducible and that urine sTNFR-2 was accurate in predicting malaria irrespective of the assay platform used. These data are in agreement with previous reports that membrane-bound TNF-α receptor 2 is expressed at higher levels on immune cells than receptor 1 and the shedding of receptor 2 is more inducible [41].

Compared to sTNFR-2 in blood and to other host biomarkers, sTNFR-2 in urine holds greater potential as a biomarker for PM. First, the concentrations of soluble TNF-α binding proteins are consistently higher in urine than in paired blood samples [34]. Their detection in urine would thus be more a sensitive approach than detection in blood. Kabyemela et al [14] and Boström et al [42] reported that IL-10 in plasma was a better predictor biomarker of maternal malaria than plasma sTNFR. On the other hand, it has been shown that IL-10—even when high in plasma—is barely detectable in matched urine samples [43]. Other plasma biomarkers that had moderate ability to diagnose occult PM infections [44] have not been assessed in urine. Second, using noninvasive urine samples increases patient compliance because sample collection is painless. Many individuals, especially those suffering from needle phobias [45, 46], will be more willing to provide urine samples than blood. Risk of exposure to blood-borne pathogens is also avoided. Third, it is interesting to note that sTNFR-2 performed well even though (1) urine samples used were not early-morning first emissions and (2) the time of the day when samples were collected was not synchronized for all participants. Sample collection was reflective of practical field settings where women present at health facilities at different times of the day.

Although only 66 participants were included, the sample size seems to be sufficient because a previous finding that sTNFR concentration was higher in urine of pregnant compared with nonpregnant women was reproduced. In addition, ROC results from ELISA data were replicated by the Luminex assay. However, a limitation of the study was the use of peripheral microscopy as gold standard because this method is known to miss some cases of PM. Placenta tissue was not available for histologic diagnosis of PM because the pregnant study participants were cross-sectionally recruited before delivery.

Because sTNFR are host biomarkers, conditions such as sepsis, which may also be associated with increase in sTNFR levels [26, 43], put into question the positive PM predictive value of sTNFR. An assessment of codetection of sTNFR-2 and the malaria parasite-specific HRP-2 was done but offered no improvement in accuracy. Urine HRP-2 performed very poorly as a malaria biomarker. The moderate accuracy of urine HRP-2 reported by Oguonu et al [47] may have resulted from the inclusion of exclusively febrile patients in that study, whereas up to 85% of participants in the present study were afebrile. Further studies are required to assess the influence of malaria in pregnancy over other pathologies on urine sTNFR levels.

CONCLUSIONS

Overall, the results of our study show that malaria in pregnancy causes significant increase in the excretion of sTNFR-1 and sTNFR-2 in urine. To the best of our knowledge, no previous studies have investigated malaria-associated changes in the urine levels of these receptors during pregnancy. We also show a positive association between peripheral parasitemia and urine sTNFR-1 and sTNFR-2, even in asymptomatic pregnant women. The present data suggest that sTNFR-2 in urine may be a useful biomarker for malaria in pregnancy, but more studies are needed to test its diagnostic accuracy in occult PM without peripheral parasitemia.

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