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SERUM CYTOKINE PROFILES IN PATIENTS WITH *PLASMODIUM VIVAX* MALARIA: A COMPARISON BETWEEN THOSE WHO PRESENTED WITH AND WITHOUT HYPERPYREXIA

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Abstract. Serum cytokine profiles in patients with *Plasmodium vivax* malaria who presented with and without hyperpyrexia were compared by a retrospective review of the medical records of the consecutive patients seen at the military hospitals near the demilitarized zone in the Republic of Korea from April 2000 through October 2001. Of 162 male patients studied, 120 (86.4%) presented with hyperpyrexia (i.e., an axillary temperature ≥ 40°C). The mean ± SEM ages of the patients with and without hyperpyrexia were 21.5 ± 0.14 and 21.9 ± 0.39 years, respectively ($P = 0.33$). The mean ± SEM concentrations of serum interleukin (IL)-6 (379.7 ± 44.1 pg/mL versus 105.4 ± 26.8 pg/mL; $P = 0.002$), IL-10 (583.4 ± 58.2 pg/mL versus 142.4 ± 39.7 pg/mL; $P = 0.0001$), and interferon-γ (312.6 ± 33.9 pg/mL versus 112.9 ± 27.1 pg/mL; $P = 0.0001$) were significantly higher in patients with hyperpyrexia compared with those without hyperpyrexia. The mean ± SEM concentrations of serum tumor necrosis factor-α were 155.5 ± 54.5 pg/mL and 109.9 ± 29.3 pg/mL ($P = 0.27$) in patients who presented with and without hyperpyrexia, respectively. Further studies are needed to examine whether serum concentrations of these cytokines also parallel their concentrations at the tissue sites of their production and action.

INTRODUCTION

As in many bacterial infections,[1] hyperpyrexia is considered a hallmark of severe illness in malaria.[2] However, the factors responsible for the development of pyrexia in general and hyperpyrexia in particular remain controversial.[3] In fact, the cause of pyrexia in malaria has been a matter of controversy for many years.[4] Unlike many gram-positive and gram-negative organisms,[5] no cell wall components of the malarial parasite have been identified as being responsible for the development of fever in malaria.[3] Instead, the current consensus on the pathogenesis of fever in malaria emphasizes the role of various pro-inflammatory cytokines derived from host's immune cells that have been induced by as yet unknown parasite-derived factors.[6,7] Tumor necrosis factor-α (TNF-α) is considered by some investigators[8,9] as being central in the pathogenesis of fever in malaria. Others have found a positive correlation between the serum concentration of TNF-α and the degree of pyrexia in malaria.[10,11] However, there is a growing body of evidence to suggest that TNF-α may not necessarily be the most important pyrogenic cytokine in humans,[12] and that TNF-α may be responsible for the maintenance, rather than the initiation, of pyrexia in proven microbial infection.[13] Therefore, we compared the cytokine profiles of patients with *Plasmodium vivax* malaria who presented with and without hyperpyrexia.

MATERIALS AND METHODS

The study population was composed of 162 consecutive patients with *P. vivax* malaria seen at military hospitals in Korea from April 2000 through October 2001. These were military hospitals situated near the demilitarized zone (DMZ) that marks the dividing line between South and North Korea. The diagnosis of *P. vivax* malaria was established in all patients by the presence of pyrexia (i.e., axillary temperature ≥ 37.0°C) and a positive malaria smear (i.e., one or more asexual forms of *P. vivax* seen after microscopic examination of 200 fields of Giemsa-stained thick (peripheral) blood films at a magnification of ×1,000).

The study protocol was reviewed and approved by the Ethics Committee and the Institutional Review Board of the participating institutions. Informed consent of the patient was not required due to retrospective nature of the study. All subjects were identified on a data collection sheet by a study number without any reference to their name and hospital number.

Axillary temperature was recorded for all patients at presentation and very two hours thereafter during the course of hospitalization. Laboratory investigations at presentations included determination of the hemoglobin level, hematocrit, total white blood cell (WBC) count, platelet count, and levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Venous blood samples were also collected for determining serum concentrations of interleukin (IL)-1, IL-4, IL-6, IL-10, IL-12, TNF-α, and interferon-γ (IFN-γ).

For the cytokine assays, venous blood samples were drawn aseptically into Vacutainer® tubes (Becton Dickinson and Company, Franklin Lakes, NJ). Serum samples were separated and aliquots were frozen at −70°C until assayed. In all cases, tests to measure serum cytokine levels were run in duplicate. The arithmetic mean of the results of both tests was considered the final result. Commercially available specific enzyme-linked immunosorbent assay kits (OptEIA™; Pharmingen, San Diego, CA) were used for the cytokine assays according to the manufacturer's instructions. For the purpose of this study, hyperpyrexia was defined as an axillary temperature ≥ 40.0°C.

Statistical analyses were done using the Wilcoxon rank sum test without any assumption about the distribution of data. Standard statistical software (SAS 6.12 version; SAS, Inc., Carey, NC) was used for all statistical analyses. Statistical significance was defined as a P value < 0.05.

RESULTS

The baseline characteristics of the study patients are shown in Table 1. All were active duty military personnel from within the catchment areas of Goyang, Paju, Yangju, Yeoncheon, and Cheolwon. All presented within 24 hours of the onset of fever when they felt that they were still febrile. The mean ± SEM of age of the patients who presented with hyperpyrexia was not significantly different from those who did not (21.5 ± 0.14 years versus 21.9 ± 0.39 years; $P = 0.33$). There were no significant differences between the two patient populations in terms of hemoglobin level (14.1 ± 0.12 g/dL versus 13.6 ± 0.29 g/dL; $P = 0.73$), hematocrit (40.4 ± 0.39% versus 39.1 ± 0.94%; $P = 0.41$), and platelet count (94.5 ± 3.8 $\times 10^9$/L versus 104.9 ± 10.5 $\times 10^9$/L; $P = 0.88$). In contrast, the hyperpyrexial patients presented with significantly lower WBC counts compared with those without hyperpyrexia (5.2 ± 0.34 $\times 10^9$/L versus 6.4 ± 0.29 $\times 10^9$/L; $P = 0.0001$). The mean ± SEM serum concentrations of ALT (50.9 ± 5.8 U/L versus 44.9 ± 8.5 U/L; $P = 0.31$), AST (45.2 ± 3.8 U/L versus 36.3 ± 4.3 U/L; $P = 0.09$), IL-1 (127.8 ± 41.7 pg/mL versus 67.1 ± 23.9 pg/mL; $P = 0.96$), IL-4 (91.4 pg/mL ± 25.6 versus 54.3 ± 18.3 pg/mL; $P = 0.38$), IL-12 (258.7 ± 67.4 pg/mL versus 393.6 ± 138.5 pg/mL; $P = 0.98$), and TNF-α (155.5 ± 54.5 pg/mL versus 109.9 ± 29.3 pg/mL; $P = 0.27$) was not significantly different in hyperpyrexial patients compared with those who did not have hyperpyrexia. In contrast, patients with hyperpyrexia presented with significantly higher concentrations of IL-6 (379.7 ± 44.1 pg/mL versus 105.4 ± 26.8 pg/mL; $P = 0.002$), IL-10 (583.4 ± 58.2 pg/mL versus 142.4 ± 39.7 pg/mL; $P = 0.0001$), and IFN-γ (312.6 pg/mL ± 33.9 versus 112.9 ± 27.1 pg/mL: $P = 0.0001$) compared with those without hyperpyrexia.

All patients received oral hydroxychloroquine sulfate (600-mg base, followed by an additional 300-mg base after six hours, and then 300-mg base again on days 2 and 3), followed by oral primaquine phosphate (15-mg base per day orally for two weeks). After discharge from the hospital, no patient included was followed-up for relapse.

TABLE 1
Baseline characteristics of male patients with *Plasmodium vivax* malaria*

Characteristics	With hyperpyrexia† (n = 120)	Without hyperpyrexia (n = 42)	P
Age (years)	21.5 ± 0.14	21.9 ± 0.39	0.33
Axillary temperature (°C)	40.2 ± 0.06	37.5 ± 0.12	<0.001
WBC count (×10⁹/L)	5.2 ± 0.34	6.4 ± 0.32	0.0001
Hemoglobin (g/dL)	14.1 ± 0.12‡	13.6 ± 0.29	0.73
Hematocrit (%)	40.4 ± 0.39‡	39.1 ± 0.94	0.41
Platelet count (×10⁹/L)	94.5 ± 3.8‡	104.9 ± 10.5	0.88
Serum ALT (U/L)	50.9 ± 5.8§	44.9 ± 8.5‡	0.31
Serum AST (U/L)	45.2 ± 3.8‡	36.3 ± 4.3‡	0.09
Serum IL-1 (pg/mL)	127.8 ± 41.7	67.1 ± 23.9	0.96
Serum IL-4 (pg/mL)	91.4 ± 25.6	54.3 ± 18.3	0.38
Serum IL-6 (pg/mL)	379.7 ± 44.1	105.4 ± 26.8	0.002
Serum IL-10 (pg/mL)	583.4 ± 58.2	142.4 ± 39.7	0.0001
Serum IL-12 (pg/mL)	258.7 ± 67.4	393.6 ± 138.5	0.98
Serum TNF-α (pg/mL)	155.5 ± 54.5	109.9 ± 29.3	0.27
Serum IFN-γ (pg/mL)	312.6 ± 33.9	112.9 ± 27.1	0.0001

* Values are the mean ± SEM. WBC = white blood cell; ALT = alanine aminotransferase; AST = aspartate aminotransferase; IL = interleukin; TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ. Normal values: WBC count = 4.0–11.0 $\times 10^9$/L; hemoglobin = 11.5–13.5 g/dL; hematocrit = 39.0–45.0%; platelet count = 150.0–450.0 $\times 10^9$/L; serum ALT = 10.0–40.0 units/L; serum AST = 10.0–42.0 units/L.
† Defined as an axillary temperature ≥ 40°C.
‡ Data not available for one patient.
§ Data not available for two patients.

DISCUSSION

The purpose of this study was to compare serum cytokine profiles of patients with *P. vivax* malaria presenting with and without hyperpyrexia. The data presented herein show that as group, the patients who presented with hyperpyrexia had significantly higher serum concentrations of IL-6, IL-10, and IFN-γ compared with those who did not present with hyperpyrexia. However, there was no significant difference between these two patient populations in terms of serum concentrations of other the cytokines tested (Table 1). Our data do not support the contention that in *P. vivax* malaria there is a positive association between the degree of pyrexia and concentration of TNF-α in serum.[10,11,14] The study design precluded us from determining whether the two patient populations differed in terms of TNF-α concentrations at the site of its production and action. However, this is highly pertinent for several reasons. First, TNF-α and other cytokines are capable of mediating critical cellular responses when present in tissues at concentrations that are far lower than their concentrations in serum.[15] Second, production of cytokines at the tissue level is considered by many to be more important than their production in the blood for the induction of signals leading to the development of fever.[12] Third, up to what extent cytokines, including TNF-α, which are large hydrophilic peptides cross the brain-blood barrier to act on the hypothalamic thermo-regulatory region is not clear.[16] Finally, the concentrations of serum TNF-α, as measured immunologically in the study subjects, may not be representative of the concentrations of the biologically active (i.e., fraction unbound with TNF-α receptors) fraction of TNF-α.[3]

Compared with those presenting without hyperpyrexia, hyperpyrexial patients presented with significantly higher serum concentrations of IL-6 and IFN-γ. Brown and others[17] have also found a positive association between the serum concentration of IFN-γ and degree of pyrexia in patients with *P. vivax* malaria. However, significantly higher serum concentrations of IFN-γ found in the hyperpyrexial compared with non-hyperpyrexial patients studied herein is somewhat baffling. This is because IFN-γ, unlike IL-6,[18] may not be intrinsically pyrogenic, and the pyrogenic properties of IFN-γ are mediated through the induction of IL-1 and TNF-α.[19] This makes it questionable whether the differences in serum concentrations of IFN-γ, as noted in this cohort study, could explain the differences in their mean presenting temperatures (Table 1), since the serum concentrations of IL-1 or TNF-α at presentation were not significantly different between hyperpyrexial and non-hyperpyrexial patients. Serum concentrations of IL-10 were significantly higher in hyperpyrexial patients compared with those without hyperpyrexia. This is not unexpected because stronger pro-inflammatory cytokine responses, as shown by the significantly higher serum concentrations of IL-6 and IFN-γ in hyperpyrexial patients, are expected to be balanced by a strong anti-inflammatory cytokine response.[20]

At presentation, the total WBC count was significantly higher in non-hyperpyrexial patients compared with that observed in patients with hyperpyrexia. The reasons for this remain unclear. Although IL-1 can cause an increase in the peripheral blood leukocyte count by mobilizing leukocytes from bone marrow,[21] IL-6 can do this by mobilizing and demarginating leukocytes from bone marrow and intravascular pool, respectively.[22] In this study, there was no significant difference between the study cohorts in terms of the serum concentration of IL-1 at presentation. Furthermore, serum IL-6 concentrations were significantly higher in hyperpyrexial group compared with those without hyperpyrexia. These again raises the possibility that these two patient populations differed in terms of concentrations of IL-1 and IL-6 at the sites of their action in bone marrow, vascular endothelia, or both.

The level of parasite density was not determined in any patient studied herein. However, it is more likely[3,23] that the levels of parasite density would be higher in patients with hyperpyrexia than those without hyperpyrexia. Although cases of minimally symptomatic *P. vivax* malaria with high levels of parasitic density, presumably due to immune tolerance resulting from repeated exposures,[10] have been reported from areas hyperendemic for malaria,[24] this is unlikely to be the case in our patients. This is because malaria is not hyperendemic in the DMZ.[25] No patient in this study had a history of a diagnosis of malaria by a physician. All patients were repeatedly asked about this during the course of hospitalization. Furthermore, all study subjects repeatedly denied having been treated for malaria at any time prior to the current illness that prompted them to seek medical attention. Although nutritional status may be relevant in the host's febrile response to infection,[26] it is considered unlikely that any difference in nutritional status between the study cohorts could explain the differences in their presenting temperature (Table 1) because they all were active duty military personnel with a standard diet. In all patients studied, the possibility of other causes of fever was excluded clinically by the physicians responsible for caring the patients. No patient received any antimicrobial agent other than those mentioned earlier. Since all study subjects were males, a practical implication of this is that it excludes the possibility of any sex-related difference in the host's febrile response[27] in infection and level of malarial parasitemia.[28]

A study conducted in Sri Lanka has conveniently examined serum levels of TNF during paroxysm in individual patients infected with *P. vivax*.[8] However, this study has addressed only one aspect of the febrile responses seen in *P. vivax* malaria, since many patients with *P. vivax* malaria, though febrile, do not develop paroxysm at any stage during the course of their illness.[29,30] More importantly, individual patients may differ in their sensitivity to a given amount of TNF.[31] Furthermore, the amount of TNF produced by patients with malaria may vary among individuals residing in the same area who have been exposed to similar parasites and with inoculation rates.[32]

Several studies[7,10,33,34] have examined serum cytokine profiles in patients with *P. vivax* malaria. However, the findings of these studies, in comparison with those of our study, are tempered with methodologic limitations. First, these studies included subjects who had previously experienced malarial attacks. These subjects were included, despite the possibility that the immune responses mounted against malarial parasites by hosts who have experienced malarial attacks may be different from those mounted by hosts lacking such experiences.[31] Second, some of these studies[10,33] did not consider possible differences between children and adults in terms of their cytokine[35] and febrile[36] responses to malarial parasites. Third, none of these studies considered the possibility of sex-related differences in the host's febrile responses in infections.[27] It is also questionable whether the findings of these studies, which were conducted in the tropical countries and had study subjects selected from individuals living indigenously in these countries, will be valid in case of *P. vivax* malaria, as seen in an indigenous population in a temperate country such as Korea. Future prospective studies should address this issue. This is important given the possibility of the existence of tropical and temperate zone types of *P. vivax*.[37]

It is also believed that the adaptive properties of *P. vivax* in different climatic conditions may have an effect on the clinical expression of *P. vivax* malaria.[38] Of note, Chai[39] and Kim[40] have already reported what they described as early and delayed onset type of *P. vivax* malaria in indigenous Korean subjects. However, apart from the rapidity of the onset of clinical disease and relapse,[41] it is not known whether the clinical expression of delayed onset *P. vivax* malaria differs from those of the early onset-type disease. Unfortunately, we could not address this issue in our study because unlike the studies of Chai[39] and Kim,[40] we did not have a subgroup of patients who had once lived in a malarious area and developed malaria at varying intervals only after they had left the endemic area. At the time of admission, the subjects included in our study had been resident in the DMZ for 12–14 months. No patient included in this study was found to have had an early- or delayed-onset type of *P. vivax* malaria when the patient's date of admission had been considered in conjunction[42] with the patient's duration of residency in the DMZ at the time of admission and the season of peak transmission of malaria. All patients denied having ever visited any geographic location outside Korea where malaria was known to be endemic. Prior to admission, no patient included in this study had ever been tested for evidence of infection by *P. vivax*. This is not unexpected because active duty servicemen in the DMZ are not routinely tested for malarial infection.

The possible effects of differences in the temporal expression of cytokines between the study subjects were considered. No patient included in this study had a temperature subsequently recorded during the course of hospitalization that was higher than that recorded on admission. All patients in the non-hyperpyrexial group repeatedly denied having ever experienced sudden onset of chills and rigors followed by sharp increases in temperature considered typical[8] of the febrile paroxysm of *P. vivax* malaria during the course of the current illness that led them to seek medical attention. By their own accounts, which may not be inaccurate,[43] it was the persistence of feverishness and body aches unassociated with any apparent daily fluctuation in body temperature that prompted them to seek medical attention. We believe, as do other investigators,[44] that the pattern of febrile illness reported by these patients on admission is not incompatible with *P. vivax* malaria. Furthermore, although considered characteristic, a considerable proportions of patients with *P. vivax* malaria do not develop febrile paroxysm at any stage during the course of their illness.[29,30]

In conclusion, male patients with *P. vivax* malaria who presented with hyperpyrexia had significantly higher serum concentrations of IL-6, IL-10, and IFN-γ compared with those without hyperpyrexia. The serum concentrations of TNF-α were not significantly different between those who presented with hyperpyrexia and those who did not. Further studies are needed to examine whether serum levels of these cytokines in patients with *P. vivax* malaria also parallel their concentrations at the tissue sites of their production and action.

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