J Parasit Dis (Jan-Mar 2019) 43(1):54–58
https://doi.org/10.1007/s12639-018-1055-2

ORIGINAL ARTICLE

Level of circulating steroid hormones in malaria and cutaneous leishmaniasis: a case control study

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Received: 12 June 2018 / Accepted: 12 November 2018 / Published online: 20 November 2018

Abstract Epidemiological and clinical studies have shown a great difference in the severity and prevalence of infectious diseases in men and women and various studies have shown that the key immunological factors are affected by sex-associated hormones. Considering the role of sex hormones in various infections, the current study aimed to determine the level of sex hormones in patients with cutaneous leishmaniasis (CL) and malaria and compare it with those of healthy controls. The survey was designed as a case–control study. Peripheral blood was collected from thirty male malaria patients, sixty patients (equal number of both sexes) with cutaneous leishmaniasis and ninety healthy subjects. Disease confirmations were done through microscopic examination of either peripheral blood smears, in case of malaria, or Giemsa-stained lesion imprint slides for CL. The level of testosterone, progesterone and estrogen were measured in malaria and CL patients along with healthy subjects, using an ELISA commercial kit. Age of participants was 18–35 years (mean 25.39 ± 4.70) for CL patients and 14–41 years (mean 27.63 ± 9.09) for malaria patients. Differences between the age of patients and the healthy subjects were insignificant. The level of testosterone in malaria patients (1.44 ± 0.12 ng/mL) was lower than control group (1.46 ± 0.06, ng/mL) but the differences were not statistically significant ($p > .05$). The concentration of testosterone in CL patients (1.49 ± 0.03 ng/mL) was higher than those of control group (1.46 ± 0.06 ng/mL), and the difference was statistically significant ($p = 0.05$). Although the concentration of estrogen and progesterone in CL patients were lower than controls, still the differences were not statistically significant ($p > 0.05$). Findings of the current study demonstrated a significant difference in the serum level of testosterone in CL patients in comparison with the healthy subjects whereas such difference was not seen in malaria patients.

Keywords Steroid hormones · Malaria · Cutaneous leishmaniasis

Introduction

Leishmaniasis is the third most important vector-borne disease among tropical diseases with 12 million annual incidences (Alvar et al. 2012). The disease is endemic in 88 countries including Iran and men are more frequently infected than women (Alvar et al. 2012; Bernin and Lotter 2014; Sarkari et al. 2012, 2010, 2016a, b; Davami et al. 2010). Malaria is yet known as one of the most common and lethal illness in tropical and subtropical areas of the world (Mohammadzadeh et al. 2014; Hatam et al. 2015; Daily 2017). Epidemiological and clinical studies have shown a great difference in the severity and prevalence of infectious diseases in men and women (Bernin and Lotter 2014; Sarkari et al. 2016a, b; Khosravani et al. 2012). Host endocrine system, affected by the immune system and the parasites. In many infective diseases, alterations in hormone levels have shown to be related to differences in cytokine profile (Baccan et al. 2011). Ability of hormones

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at influencing the immunological responses in parasitic infections has drawn the attention of researchers in recent years (Escobedo et al. 2005). The cells of immune system possess androgen, estrogen and progesterone receptors. Accordingly, the outcome of various diseases, including leishmaniasis can be affected by gender. Based on the epidemiological studies, males are more susceptible to leishmaniasis (Snider et al. 2009). Testosterone is considered to be the most important androgen in men and progesterone in women as immunosuppressive hormones; which both reduce the activity of natural killer cells (Faust et al. 1999; Lotter et al. 2013).

Various studies have shown that key immunological factors are affected by gender and pregnancy (Robinson and Klein 2012; Ghazeeri et al. 2011). Therefore, the effect of sex and the sensitivity and resistance to some of the parasitic infections, can be attributed to the effect of hormones. Some epidemiologic studies have, however, reported the presence of sex differences in parasitic infection among humans (Bernin and Lotter 2014; Lezama-Davila et al. 2007). Because male and female are different in immunological responses against infectious diseases, therefore more studies on this concept are needed. Considering the role of sex hormones in various infections, the aim of this study was to determine the level of sex hormones in males and females in patients with cutaneous leishmaniasis and malaria and to compare it with those of healthy individuals.

Material and methods

Sampling

The survey was designed as a case–control study. Fresh blood samples were taken from thirty male malaria patients in Hormozgan province, South of Iran, sixty patients (thirty cases in each gender) with cutaneous leishmaniasis and ninety healthy age-matched controls in Fars province, southwest of Iran. From each participant, 5 mL of fresh venous blood was collected in anticoagulant-containing tubes (Pishtaz Teb Zaman Diagnostics, Iran). Sera were separated after clotting, by centrifugation for 10 min at 800 g. While keeping the cold chain, the samples were transferred to the immunoparasitology laboratory at Shiraz University of Medical Sciences (SUMS), where they were stored at − 20 °C until use. Formal informed consent was obtained from each participants and the study was approved by the ethic committee of Shiraz University of Medical Sciences (SUMS). In females' subjects, menstrual period of patients and control was considered during sample collection. Dismissal criteria included history of endocrine disorders, hormone therapy, patients taking immunosuppressive drugs and pregnancy. Sera were isolated from the blood and kept frozen at − 20 °C until use.

Malaria and cutaneous leishmaniasis confirmation

Malaria and CL confirmation were done through microscopic examination of both thick and thin peripheral blood smears, in case of malaria, or Giemsa-stained skin lesion imprint slides for CL. In both cases Giemsa stained samples were evaluated microscopically at 100 × oil immersion for detection of *Plasmodium* or *Leishmania* parasites.

Measurements of testosterone, progesterone and estrogen hormones

The serum levels of testosterone, progesterone and estrogen were measured in malaria and CL patients along with healthy subjects, using an ELISA commercial Kit (Ideal Tashkhis Atieh Co., Iran). The measurements were operated according to the manufacturer's instructions. Briefly, standard specimen and sera samples (which were kept at − 20 °C) were placed in ELISA plate wells. Then, the enzyme conjugate solution was added to each well. Further, the biotin conjugate was added to each well, and after adding the substrate and the stopping solution, the optical density was measured at 450 nm.

Statistical analyses

Collected data were analyzed, using SPSS (Ver. 18) software. Each hormone concentrations in controls and patients were compared using Student's t test. Mean differences were considered statistically significant if p was < 0.05.

Results

Mean ages of participants for malaria patients and controls were 25.39 (± 4.70) and 27.03 (± 6.21), respectively. Also mean ages of leishmaniasis patients and controls were 25.90 (± 5.10) and 27.03 (± 6.21), respectively. The level of testosterone in malaria patients (1.44 ± 0.12 ng/mL) was lower than control group (1.46 ± 0.06, ng/mL) but the differences were not statistically significant ($p > 0.05$) (Table 1). The concentration of testosterone in CL patients was higher than those of control group, and this difference was fairly statistically significant ($p = 0.05$). Although the concentration of estrogen and progesterone in CL patients were lower than those of control counterparts, however the differences were not statistically significant ($p > 0.05$). Table 2 shows the mean levels of studied hormones in CL patients and healthy controls.

Table 1 Mean testosterone levels in malaria patients and healthy controls

	Malaria patients	Healthy controls	*p* Value
Testosterone (ng/mL) mean ± SD	1.44 ± 0.12	1.46 ± 0.06	0.6

Table 2 Mean hormone levels in cutaneous leishmaniasis patients and controls

	Female		Male		*p* Value
	CL patients	Healthy controls	CL patients	Healthy controls	
Estrogen (μIU/mL)	0.57 ± 0.46	0.64 ± 0.57	–	–	0.5
Progesterone (μIU/mL)	2.35 ± 0.91	2.43 ± 0.79	–	–	0.7
Testosterone (ng/mL) mean ± SD	–	–	1.49 ± 0.03	1.46 ± 0.06	0.05

Discussion

A better understanding of gender-dependent differences in human disease will allow for the design and development of more efficacious treatments and vaccination programs (Snider et al. 2009). In recent years there has been strong speculation that sex hormones might play some role in resistance during parasite infections. A few epidemiologic studies have also established the contribution of sex hormones in infectious diseases, including malaria and cutaneous leishmaniasis (Giefing-Kröll et al. 2015; Klein 2004; Kurtis et al. 2001). There are evidence that prevalence, intensity and responses to vaccines in some of parasitic infections, are higher in males in comparisons with females (Klein et al. 2008). Sex hormones cause modulation of immune responses and influence the outcome of parasitic infections (Benten et al. 1997). Testosterone is thought to act as an anti-inflammatory factor while estrogen is more pro-inflammatory in nature (Klein 2004). High testosterone concentrations causing increase competition and aggressiveness and increase the susceptibility to parasites in males (Folstad and Karter 1992). In the present study, testosterone concentration in malaria patients was lower than healthy controls, but the differences were insignificant. In accordance with this study, Abdagalil et al. demonstrated a decreased level of testosterone in heavily-infected malaria male patients. Authors suggested that testosterone levels decrease significantly with the increasing in the degree of parasitemia (Abdagalil and ElBagir 2009). To find out the hormonal mechanisms that mediate sex dissimilarities in susceptibility to malaria infection Cernetich et al. infected intact and gonadectomized C57BL/6 mice with *Plasmodium chabaudi* and found that males were 3.5 times more likely to die from malaria infection than females (Cernetich et al. 2006). Moreover, male mice exhibited more severe anemia and higher peak parasitemia than females. It was also found that testosterone decreases IFN-γ and regulatory T-cell mRNA expression in male C57BL/6 mice at the height of parasitemia (Cernetich et al. 2006). In another study, castration of male C57BL/10 mice infected with *P. chabaudi* causes resistance to the disease and treatment with testosterone improved of infected female C57BL/10 mice (Benten et al. 1992, 1997). Testosterone and other gonadal factors restrict the efficacy of genes controlling resistance to *P. chabaudi* malaria (Wunderlich et al. 1991). It has also been shown that sex hormones modulate the immune responses to *P. berghei* in CBA/Ca mice (Legorreta-Herrera et al. 2015). In leishmaniasis although some epidemiological study showed that the disease are more severe in males (Lezama-Davila et al. 2007; Muñoz and Davies 2006), but a study in Afghanistan showed that females are more susceptible to lesions and scars due to *L. tropica* infection (Reithinger et al. 2003). Travi believes that in leishmaniasis patients testosterone and other androgens stimulate T-helper 2 (Th2) response and change the course of parasitic infection (Travi et al. 2002). Pourmohammadi et al. reported that in CL patients there is no gender difference between the glucantime sensitive and resistant cases (Pourmohammadi et al. 2011). In a study by Ferede et al. prevalence of VL-malaria coinfection was higher in males than females but the difference was not significant (Ferede and Diro 2017). Immune cells possess receptors for sex steroid hormones such as testosterone, estrogens and progesterone (Bouman et al. 2005). Therefore steroid hormones could affect the outcomes of leishmaniasis (Pandey et al. 2014). Estradiol that is the most common type of estrogen, involved in lesion development in leishmaniasis. Baccan showed that individual with localized cutaneous leishmaniasis show lower plasma levels of DHEA-S, prolactin and testosterone in comparison with controls group, whilst levels of cortisol and estradiol were similar between patients and controls (Baccan et al. 2011). Unlike malaria, in this study testosterone level in

CL patients was higher than healthy controls, but still the differences in the levels were statistically insignificant. Estrogen and progesterone levels of CL patients were lower than healthy controls but the difference again was not significant. A gender-related difference in CL was reported by Travi et al., where prepubertal hamster had smaller and/or less severe lesions than adult male animals (Travi et al. 2002). Based on Travi observation, treatment of female hamsters with testosterone causes larger lesions, while treatment of male hamsters with estrogen has little effects on disease outcome (Travi et al. 2002). In rheumatoid arthritis effects of estrogen seems to be bimodal; pro-inflammatory at pharmacological concentrations and anti-inflammatory in physiologic concentrations (Cutolo et al. 2004).

In conclusion, in our study no obvious changes in sex hormone in patients infected with malaria, in comparison to the healthy subjects were seen whereas associations between disease and testosterone levels in CL patients were significant. It should be considered that the outcome of the parasitic infections appears to be linked to the genetic background of the hosts, pathogenicity and virulence of the parasite, and the immunity status as well as sex hormone levels in the hosts. More studies with larger sample size along with calculation of parasitemia are needed to appropriately address the possibility of association of sex hormones and disease severity in malaria and CL.

Acknowledgements The results described in this paper were part of MD thesis of Najme Shakouri. The study was financially supported by the office of vice-chancellor for research of Shiraz University of Medical Sciences (Grant No. 94-01-43-9603).

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