Increased total antioxidant capacity in renal tissue of female BWF1 mice infected with \textit{Plasmodium chabaudi}

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\textbf{INTRODUCTION}

Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune disease whose aetiology and pathogenesis are incompletely understood (Monova \textit{et al.}, 2011). Because of its multifactorial etiology, which includes genetic, hormonal, and environmental triggers, the molecular mechanisms underlying this disease remains largely unknown. Free radical-mediated reactions that leads to oxidative stress have recently drawn considerable attention as a potential mechanism of the pathogenesis of SLE (Kurien \textit{et al.}, 2006). Cells, tissues, and body fluids are equipped with powerful antioxidant defense systems that help counteract oxidative challenge (Sies, 2007). When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease. Consequently, the maintenance of adequate antioxidant levels is essential to prevent or even manage a great number of disease conditions (Kusano and Ferrari, 2008). The term total antioxidant capacity (TAC) is now well-accepted by biochemists and it is formulated as the “cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants” (Ghiselli \textit{et al.}, 2000). In many different pathophysiological conditions, TAC could be a reliable biomarker of diagnostics and prognostics (Kusano and Ferrari, 2008).

Urine analysis provides a highly imperative diagnostic tool for several disease conditions especially renal diseases (Gou \textit{et al.}, 2013), urinary biomarkers were frequently used to follow up the different stages of disease progression in human (Reyes-Thomas \textit{et al.}, 2011). Renal involvement in patients with SLE in the form of severe lupus nephritis (LN) is associated with a significant change in the urine biochemistry (Smith and Beresford, 2016).

Tropical infections, particularly malaria, have a mysterious relationship with SLE (Clatworthy \textit{et al.}, 2007). Epidemiological studies have revealed that SLE is rarely observed in rural tropical areas of Africa and Asia, where malarial infection is prevalent (Minaur \textit{et al.}, 2004). Therefore, it has been hypothesized that SLE-susceptibility genes are beneficial in controlling severe malaria but promote inflammation in the absence of malaria (Greenwood and Corrah, 2001). Greenwood \textit{et al.} (1970) described a higher survival rate in young lupus-prone mice infected with \textit{Plasmodium berghei yoelii}. In our previous work, we have confirmed that both the kidney and the liver have an important role during infection of female BWF1 lupus mice with \textit{P. chabaudi} (Al-Quraishy \textit{et al.}, 2013). On the other hand, the protein concentration in urine was elevated in female BWF1 mice after live \textit{P. chabaudi} infection (Abdel-maksoud \textit{et al.}, 2016). Hence, the current study aimed to more deeply investigate the possible role of malarial infection
on TAC in both kidney and liver and the changes in urine biochemistry induced by malaria in SLE murine model.

MATERIALS AND METHODS

Animals: A total of 30 female BWF1 29-week-old mice were purchased from Jackson Laboratory (Bar Harbor, USA) and maintained and monitored in an environment which is specific pathogen-free (SPF). All animal procedures were performed in accordance with the standards set out in the Guidelines for the Care and Use of Experimental Animals issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Animal Ethics Committee at King Saud University. All animals were allowed to acclimatize in plastic cages inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard laboratory conditions, fed a diet of standard commercial pellets and given water ad libitum.

Malarial infection: Female BWF1 mice (30-week-old) were infected by an intraperitoneal (i.p) injection of $10^6$ parasitized erythrocytes obtained from an infected mouse of the same strain as previously described (Hentati et al., 1994). Experimental animals were divided into three groups (10 mice/group) as follows: group (I) Control group (lupus uninfected with P. chabaudi); group (II) live P. chabaudi-infected group (Lupus + live P. chabaudi infection); and group (III) irradiated P. chabaudi-infected group (Lupus + irradiated P. chabaudi infection). Group III was infected i.p. with $10^6$ gamma-irradiated RBCs infected with P. chabaudi. Prior to injection, the blood cells were exposed to a dose of 200 Gy gamma-radiation from a Gamma Cell 200 Irradiator (Atomic Energy of Canada, Ltd., Ottawa, Canada) utilizing a $\gamma_{60}$ source located at the Research Centre of the College of Science, King Saud University, Saudi Arabia. All animals were sacrificed at day 14 post-infection.

Sample collection for urine parameters analysis: 24-hour urine collections were obtained in metabolic cages (Tecniplast®) that allows their quantitative and reliable collection over 24 hour periods. Urine glucose level was determined by using Glucose Assay kits (ab65333, abcam, UK) according to the instructions of the manufacturer. Absorbance was measured with an Ultrospec 2000 U/V spectrophotometer (Amersham Pharmacia Biotech, Cambridge, England). The color developed was measured at 570 nm. For ketones and blood detection in urine, a Combur 10 Test® (Roch Diagnostics GmbH, Mannheim, Germany) were used.

Sample collection for TAC determination in kidney and liver: For TAC investigation, parts of liver and kidney were weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 500 g for 10 min at 4°C. The supernatant (10%) was used for determination of TAC using commercial kits (Biovision, Milpitas, California, USA) according to the manufacturer’s instructions. The color developed was measured at 570 nm.

Statistical analysis: Prior to further statistical analysis, the data were tested for normality using the Anderson-Darling test, as well as for homogeneity variances. The data was normally distributed and is expressed as the mean ± standard error of the mean (SEM). Significant differences among the groups were analysed by one-way ANOVA followed by Bonferroni’s test for multiple comparisons using PRISM statistical software (GraphPad Software). The data was also reanalysed by one- or two-way ANOVA followed by Tukey’s post-test using SPSS software, version 17. Differences were considered statistically significant at $P<0.05$

RESULTS AND DISCUSSION

Decreased glucose concentration in urine samples of live P. chabaudi infected mice: Concentration of glucose in urine can be used as an indicator for several pathological conditions (Bekhof et al., 2015). Renal involvement in patients with SLE in the form of severe lupus nephritis is associated with a significant change in the urine biochemistry (Reyes-Thomas et al., 2011). Here, BWF1 lupus mice has exhibited an elevated level of glucosuria and this can be considered as a consequence of the lupus-associated abnormal kidney functioning. live P. chabaudi infection has resulted in a significant ($P<0.05$) decrease in the level of glucose in urine samples of lupus mice in comparison to the control (fig. 1). This is in agree with the results obtained by Al-Quraishy et al (2013) which clarified a partial improvement in the lupus-associated renal pathology after live P. chabaudi infection in BWF1 lupus mice. On the
contrary, gamma irradiated *P. chabaudi* – infected group has shown a slight non-significant decreased level of glucose in urine in comparison to the control and this effect of gamma irradiation was elucidated before (Abdel-Maksoud *et al.*, 2016).

**Increased concentration of ketone bodies in urine samples of live *P. chabaudi* infected mice:** Ketones in the urine (ketonuria) can often be detected in high levels during many metabolic disturbances. If for any reason the body cannot get enough glucose for energy it will switch to using body fats, resulting in an increase in ketone production making them detectable in the urine. In the current study, live *P. chabaudi* infection has resulted in a significant (*P*<0.05) increase in the level of ketone bodies in the urine samples of lupus mice in comparison to the control (Fig.2). This effect may be attributed to the malaria associated anorexia which is a well-known symptom of malaria infection (Bartoloni and Zammarchi, 2012). Conversely, gamma irradiated *P. chabaudi* – infected group has shown a slight non-significant increased level of ketone bodies in the urine samples of lupus mice in comparison to the control. However, when compared to the live *P. chabaudi* infection, gamma irradiated *P. chabaudi* – infected group has shown a significant (*P*<0.05) decrease in the level of ketone bodies in the urine samples of this group of mice.

**Live *P. chabaudi* infection is associated with increased hematuria:** In the current study, live *P. chabaudi* infection had significantly (*P*<0.05) increased the level of hematuria in the urine samples of lupus mice infected with live *P. chabaudi* in comparison to the control (Fig.3). Tobón Castaño *et al* (2010) have reported that the dark urine seen in malaria-infected patients is related to the bilirubinuria, hematuria and proteinuria, which can be early indicators of malaria infection. For the gamma irradiated *P. chabaudi* – infected group, no significant change could be detected in

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**Figure 1:** Effect of live or gamma-irradiated *P. chabaudi* infection on ketonuria.

**Figure 2:** Effect of live or gamma-irradiated *P. chabaudi* infection on hematuria.

**Figure 3:** Effect of live or gamma-irradiated *P. chabaudi* infection on TAC level in kidney.

**Figure 4:** Effect of live or gamma-irradiated *P. chabaudi* infection on TAC level in liver.
the level of hematuria in the urine samples of this group of mice in comparison to the control. However, when compared to the live *P. chabaudi* infection, gamma irradiated *P. chabaudi*—infected group has shown a significant (P<0.05) decrease in the level of hematuria in the urine samples of this group of mice.

**Total antioxidant capacity has been increased in renal tissue with a decrease in the hepatic tissue after live *P. chabaudi* infection:** Total antioxidant capacity (TAC) is considered as a biomarker of disease in many different pathophysiological conditions (Kusano and Ferrari, 2008). Many studies have illustrated that anti-oxidative capacity plays an important role in the severity of SLE (Gaál et al., 2016). In the current study, live *P. chabaudi* infection has resulted in a significant (P<0.05) increase in the level of total antioxidant capacity in the renal tissue of lupus mice in comparison to the control (Fig.4). Additionally, gamma irradiated *P. chabaudi*—infected group has shown a slight non-significant increased level of TAC in the renal tissue of lupus mice in comparison to the control. These findings are in agree with that obtained in our previous study which showed an increased level of reduced glutathione (GSH) in renal tissue of BWF1 lupus mice following experimental infection with live *P. chabaudi* (Al-Quraishy et al., 2013). Conversely, in liver, live *P. chabaudi* infection has resulted in a significant (P<0.05) decrease in the level of total antioxidant capacity in the hepatic tissue of lupus mice in comparison to the control (Fig.5). Additionally, gamma irradiated *P. chabaudi*—infected group has shown a slight non-significant decreased level of TAC in the hepatic tissue of lupus mice in comparison to the control. As reported before, Oxidative stress is associated with liver damage in SLE patients (Lozovoy et al., 2011). Taken together, our data illustrates that infecting BWF1 lupus mice with live, but not gamma irradiated *P. chabaudi*, have several consequences on the urine biochemistry and the TAC level in both kidney and liver.

**Conflicts of interest:**
The authors declare no conflicts of interest.

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