The Effect of Brucellosis on Lipid Profile and Oxidant-Antioxidants Status

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Abstract

The activation of inflammatory cells, the release of their mediators, and the excessive production of free radicals may affect circulating lipids, but no evidence supports a role for peroxidation in the pathogenesis of Brucellosis disease. The aim of this work is to study the effect of Brucellosis on lipid profile concentration and oxidant-antioxidant status. We studied 20 Brucellosis patients (18 Females and 2 males) and 15 healthy controls (age average from 16 to 60 years old). Significant differences were noted between the serum lipids of Brucellosis patients and control group. Mean total cholesterol and low density lipoprotein cholesterol (LDL-cholesterol) concentrations were higher in patients than in control group (mean ± SE 197.05 ± 44.7, 165 ± 37.6). ($P \leq 0.004$, $P \leq 1.59 \times 10^{-11}$) respectively. Whereas, high density lipoprotein cholesterol (HDL-cholesterol) and triglyceride show significant lower concentration in patients than control groups (mean ± SE 15.12 ± 3.4 , 81.22 ± 18.45) . ($P \leq 9 \times 10^{-6}$, $P \leq 9.3 \times 10^{-7}$) respectively. Table l , Figure 1.While circulating concentration of glutathione (GSH), soluble antioxidants were higher in Brucellosis patients than in control groups (mean ± SE 2.49 ± 0.4). Additionally, increased oxidative stress was observed in the serum of patients with Brucellosis as evidenced by higher malondialdehyde (MDA) concentrations than in control groups (mean ± SE 270 ± 9) (Figure 2, Table 1). In conclusion. Disturbances in the lipid profile, and oxidant-antioxidant status occur in Brucellosis patients which may increase incidence of metabolic and cardiovascular events.

Key words: Brucellosis disease, Lipid profile, oxidant-antioxidant status.

الخلاصة

ان تنشيط الخلايا الالتهابية وافراز مسبباتها وتكوين مزيد من الجذور الحرة يؤدي الى التاثير على دهون الدم لكن لا يوجد أي دليل في علم الامراض يدعم عملية فوق الاكسدة (Peroxidation) عند مرضى الحمى الراجعة او حمى مالطا. لدراسة تاثير الحمى الراجعة على تركيز مكونات دهون و عوامل الاكسدة و مضادات الاكسدة. تم دراسة 20 مريض من مرضى الحمى الراجعة (18 من الاناث و 2 من الذكور تتراوح اعمارهم بين 16 الى 60 سنة) و 15 من الاشخاص الاصحاء كمرجع . لوحظ عدة اختلافات ظاهرية في تركيز مصل الدهون عند مرضى الحمى الراجعة بالمقارنة مع الاشخاص الاصحاء حيث وجد ان تركيز مصل الكوليستيرول الكلي والجزئي LDL- كوليستيرول عالي نسبيا عند مرضى الحمى الراجعة بالمقارنة مع الاشخاص الاصحاء (mean ± SE 197.05 ± 44.7, 165 ± 37.6) بالتعاقب ($P \leq 1.59 \times 10^{-11}$, $P \leq 0.004$) بالتعاقب. بينما اظهر تركيز الـ HDL – كوليستيرول والترايكليسيرايد قلة واضحة و مهمة عند مرضى الحمى الراجعة بالمقارنة مع الاشخاص الاصحاء (mean ± SE 15.12 ± 3.4 , 81.22 ± 18.45) ($P \leq 9 \times 10^{-6}$, $P \leq 9.3 \times 10^{-7}$) بالتعاقب. الجدول 1 والرسم 1. من جانب آخر وجد ان تركيز مضاد الاكسدة (Glutathione) عالي عند مرضى الحمى الراجعة (mean ± SE 2.49 ± 0.4) بالاضافة الى زيادة ملحوظة في تركيز المؤكسد (Malondialdehyde) اكثر مما في الاشخاص الاصحاء (mean ± SE 270 ± 9) (الرسم 2 والجدول 1).لمناقشة هذا الموضوع اظهرت الدراسة اختلاف و عدم انتظام نسبة الدهون والمؤكسدات و مضادات الاكسدة عند مرضى الحمى الراجعة مما يؤدي الى زيادة مؤثرة في التأيض وتصلب الشرايين.

Introduction

Brucellosis was firstly described by Bruce in 1887 with the discovery of the bacterial species subsequently named *Brucella melitensis*. It is a type of febrile illness characterized by regular remissions or intermissions has been recognized in the Mediterranean region for centuries. Many names have been applied to it, often relating to the locations in which it was particularly prevalent. (Malta fever, Mediterranean fever, Gibraltar fever or Rock fever and Undulant fever) are probably the best known[1,2]. Brucellosis is the major bacterial zoonosis and an important cause of a serious debilitating disease in human, and abortion and sterility in domestic animals. It is classified into two subdivision of proteobacteria, (small gram-negative and facultative intracellular pathogens) that can multiply within professional and non professional phagocytes [3,4]. The genus brucella consists of seven species according to antigenic variation and primary host, B.melitensis (sheep and goats), B. suis (hogs), B. abortus (cattle), B. ovis (sheep), B. canis (dogs), B. neotanac (wood rats), and B. maris (marine mammals)[5].

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In contrast to other intracellular pathogens, brucella species do not produce exotoxins, antiphagocytic capsules, thick cell wall, resistance forms or fimbriae and do not show antigenic variation[6]. A key aspect of the virulence of brucella species is their ability to proliferate within professional and non professional phagocytic host cells, therefore successfully by passing the bactericidal effects of phagocytes. Their virulence and chronic infections are thought to be due to their ability to avoid the killing mechanisms within host cells [7,8]. Lipid profiles include serum cholesterol, serum triglycerides, the fractions of break up of various cholesterol like low density lipoprotein, cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol), and the ratio of LDL/HDL[10]. Total cholesterol has been found to correlate with cardiovascular mortality in 30-50 years age group. Cardiovascular mortality increases 9% for each 10mg/dl increase in total cholesterol over the base line value of l80mg/dl.HDL-cholesterol is a good cholesterol in that of cardiovascular disease decrease with increase of HDL[11]. Triglyceride level is a risk factor independent of the cholesterol level, triglycerides are important as risk factors only if they are not part of the chylomicron fraction[12]. Lipid profile is Known to alter in patients with sever sepsis, but few studies regarding the status of lipid levels in brucellosis are available[13]. Glutathione peroxidase are major enzymes that remove hydrogen peroxide generated by SOD in cytosol and mitochondria by oxidizing the tripeptide glutathione (GSH) in to its oxidized form (GSSG). The glutathione peroxidase that removes hydrogen peroxide contains selenium (essential for catalytic function) at its active site[14].

Subjects and Methods

Twenty patients (18 Females and 2 males, age average from 16 to 60 years old) with brucellosis were recruited from the clinic of Al-Khadmia Teaching Hospital. The diagnosis of the disease was based on standard clinical, histological features, and serological test which is established by positive rose Bengal plate agglutination test with antibody titer more than 1/320[15]. The severity of the disease was evaluated by the severity of abdominal pain accompanied by nausea and vomiting[16]. Gastrointestinal symptoms are noted in 40% of patients with brucellosis with anorexia, weight loss and hepatosplenomegaly are the most common. History of fever generalized myalgia and arthralgia, low back pain and sweating [17]. Study was also conducted on fifteen apparently healthy individual as a control groups, blood samples were collected after subjects had fasted for 12 hours overnight, serum was separated immediately by low speed centrifugation and stored pending analysis.

Lipid profile analysis

Serum concentration of total cholesterol and triglycerides were measured enzymatically with a commercial kit (boehringer, Mannheim montreal). According to the following principle [18]:

The cholesterol present in the sample originates a coloured complex.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-cholestadien} + H_2O_2$$

$$2\,H_2O_2 + \text{phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4\,H_2O$$

Triglyceride was measured by GPO-POD enzymatic colorimetric method as the following principle[19]:

$$\text{Triglyceride} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycero kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

HDL-cholesterol was measured after precipitation of very low density lipoprotein (VLDL) and LDL-cholesterol with phosphotungstic acid, LDL-cholesterol measured by Friede Wald equation as the following:

LDL-cholesterol = total cholesterol – (HDL + Trigly/5)

When triglyceride levels are less than 400 mg/dl[20].

Oxidant and antioxidant analysis

Serum free malondialdehyde concentrations were measured according to a modified method of Chirico (1994) [21]. Proteins were first precipitated with a 10% $NaSO_4$ solution. The protein free supernatant was then reacted with an isovolume of a 5% thiobarbiuric acid solution at 95°C for 30 min. after the reaction component were cooled to room temperature, pink chromagene was extracted with n-butanol then dried over a stream of nitrogen at 37°C.

The dry extract was then resuspended in a mobile phase of KH_2PO_4 – methanol (70:30) before malondialdehyde detection by HPLC [21].

Glutathione was measured by Titze's technique [14]. According to this equation:

$$2GSH + H_2O_2 \xrightarrow{GSH-PX} GSSG + 2H_2O$$

Statistical analysis

All values were expressed as mean ± SE. statistical differences were assessed by student's two tailed t-test. P. values ≤0.05 were considered significant.

Results

Lipid profile

Significant differences were noted between the serum lipids of patients with brucellosis and control group. Where mean total serum cholesterol concentration was higher in patients than in control group. Total cholesterol was characterized by higher LDL-cholesterol in brucellosis patients (mean ± SE 197.05 ± 44.7, 165 ± 37.6) ($P \leq 0.004$, $P \leq 1.59 \times 10^{-11}$ respectively) with a significant lower HDL-cholesterol and triglyceride concentration (mean ± SE 15.12 ± 3.4, 81.22 ±18.45) ($P \leq 9 \times 10^{-6}$, $P \leq 9.3 \times 10^{-7}$ respectively). (Table 1 , Figure 1).

Table 1 : Mean±SE (mg/dl) of serum lipid profile (total cholesterol, LDL-cholesterol, Triglyceride, HDL-cholesterol, Malondialdehyde and Glutathione) levels (nmol/l) and LDL/HDL ratio in patients with brucellosis and control groups.

	Control n=15		**Patients n=20**		
variable	**Mean**	**± SE**	**Mean**	**± SE**	**P**
Total cholesterol	190.45	± 4.3	197.05	± 44.7	≤ 0.004
Triglyceride	135.1	± 30.7	81.22	± 18.45	$\leq 9 \times 10^{-6}$
LDL-cholesterol	102.3	± 23.25	165.45	± 37.6	$\leq 1.59 \times 10^{-11}$
HDL-cholesterol	36.6	± 8.3	15.12	± 3.4	$\leq 9.3 \times 10^{-7}$
LDL/HDL ratio	2.79		10.9		≤ 0.001
MDA	76	± 17.5	270	± 9	≤ 0.001
GSH	1.9	± 0.27	2.49	± 0.4	≤ 0.005

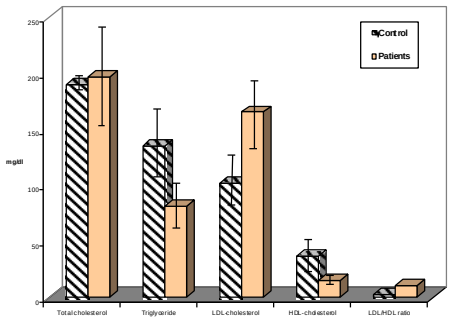

Figure1: Mean (±SE) serum lipid profile (total cholesterol, LDL-cholesterol, Triglyceride, and HDL-cholesterol) levels and LDL/HDL ratio in fasting Brucellosis patients (n= 20) and control groups (n= 15) the deference is significant.

Oxidant- antioxidant status

Circulating concentrations of glutathione, a key endogenous soluble antioxidant, were higher in patients with brucellosis than in control groups (mean ± SE 2.49 ± 0.4). Moreover, increased oxidative stress was observed in brucellosis patients as evidenced by higher malondialdehyde concentrations than in the control group (mean ± SE 270 ± 9). (Table 1, Figure 2).

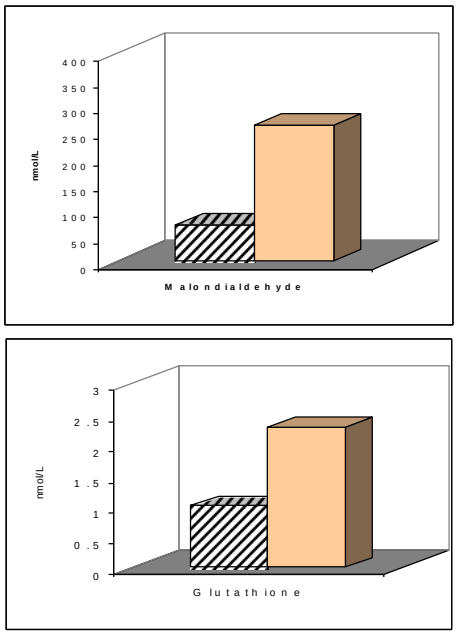

Figure 2: Mean (±SE) Malondialdehyde and Glutathione concentration in serum of patients with Brucellosis disease (n=20) and control groups (n=15) the differences are significant P< 0.005 for glutathione, and p<0.001 for Malondialdehyde.

Discussion

The present study identified important alterations in the lipid profile and oxidant-antioxidants status in patients with brucellosis compared with healthy control groups. Our data documented abnormally high serum concentrations of total cholesterol and LDL-cholesterol in patients with brucellosis, with concomitant decrease in triglyceride and HDL-cholesterol The altered oxidant- antioxidant status of brucellosis patients was shown by higher glutathione concentration and higher plasma malondialdehyde concentrations than in control groups. This last finding is consistent with abnormal lipid peroxidation in the circulation of Crohn's disease patients [13].Yet the little information is available on the plasma lipid profile and lipoprotein concentrations in patients with brucellosis. Although our findings disclose various changes in these biochemical indexes, no obvious associations were noted between these disturbances and selected clinical aspects of the patients, such as disease activity, site of disease, and current medications. Further more, none of our patients smoked or consumed alcohol, which are the two factors that may influence antioxidant and lipoprotein status. At this time, it's uncertain whether the hypercholesterolemia noted in brucellosis patients results from a consequence of high carbohydrate consumption. Similarly, further work is needed to explore whether the hypotriglyceridemia in patients results from intestinal malabsorption or malnutrition[23, 24].The excessive local production of soluble mediators from activated monocytes and polymorphonuclear leukocytes has been implicated in mediating the tissues injury [25]. Important among these mediators are oxygen free radicals. The chronic inflammatory cells promote an imbalance between oxidant and antioxidant mechanisms at the tissue level and may even compromise circulating antioxidant concentrations[26,27].However, children with CD (crohn's disease) were reported **to** have higher plasma antioxidant concentrations than healthy children[28]. In the present study, glutathione is an important intracellular antioxidant, show significant increase in patients than in control groups, consistent with other reports in adults with CD [29,30].These in consistencies in reports of circulating antioxidants to date may be due to the patient's degree of inflammation, the patient's medication and supplement use, enteric losses, altered mobilization as a result of inflamed mucosa, malabsorption and decrease nutrient

intake. Glutathione is tripeptide helps to detoxify free radicals, peroxides and electrophilic compounds of endogenous and exogenous origin [31,32].More ever ,serum glutathione level was higher in our patients than in control groups. Additional investigation is needed to verify whether the high values of glutathione constitute an adaptive to extrapolate from an experimental model[33]. These high concentrations of glutathione may help prevent oxidation of α-tocopherol, and preserve ascorbic acid concentration[34].Free radicals are known to occur as natural byproducts under physiologic conditions. However, their over production has been implicated in the pathogenesis of gut inflammation and intestinal injury in inflammatory bowel disease[28].Oxyradical induced cytotoxicity gives rise to lipid peroxidation through the reaction of free radicals and peroxides with fats in cellular membranes, resulting in malondialdehyde formation[35]. Our patients had significantly higher circulating malondialdehyde concentrations than did control groups. It is unclear whether the excess serum malondialdehyde was generated in the patient's blood or was produced by the inflamed intestine and translocated in to the circulation. The presence of malondialdehyde in the circulation may explain the increased formation of glutathione as a mean of preventing oxidative damage. Moreover, was showed that proinflamatory cytokines can adversely affect lipoprotein metabolism. For example, tumor necrosis factor α and interlukin-6 were shown to affect intestinal fat handling and lipid metabolism[32, 35].Nevertheless, our data failed to show an effect of disease activity on these indexes, likely because of the relatively small number of patients, further studies are required in which these indexes are analyzed serially in brucellosis patients over time.In conclusion we found substantial abnormalities in the concentrations of plasma lipids, malondialdehyde, and antioxidants of brucellosis patients. Moreover, the presence of excessive lipid peroxidation strategies should be elaborated to bolster the antioxidants system in patients with brucellosis. From our results in this study we suggested that brucellosis patients had high risk to atherosclerosis due to the significant increase in plasma total cholesterol, LDL-cholesterol and high level of LDL/HDL ratio, the three indexes of high risk cholesterol mortality. According to the result of this study we can concluded that patients with brucellosis may have an increasing incidence of metabolic and cardiovascular events.Further investigation is required to elucidate the mechanisms involved in aforementioned abnormalities.

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