Immunological Study of Febrile Fever: Serum Sialic Acid, Immunoglobulin Levels (IgA, IgG and IgM), Complement Factors (C3 and C4) in Patients with Typhoid Fever and Brucellosis

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ABSTRACT:

BACKGROUND:

Typhoid fever and brucellosis are frequent causes of bloodstream infections in many countries. The diagnosis of these infections is challenging because they can have diverse clinical manifestations with symptoms that overlap with a wide spectrum of other diseases. However, neither Widal nor Rose-Bengal agglutination assays are sufficiently sensitive, specific, or practical in areas of endemicity .In this study, it was undertaken to determine the sensitivity and specificity of clinical application of sialic acid in the evaluation of humoral immune response in patients with typhoid fever and brucellosis. Furthermore, the role of sialic acid (SA) was investigated as a possible biological marker and to assist diagnosis of disease.

METHODS:

Serum samples from 35 patients with typhoid fever, 35 patients with brucellosis, and 60 healthy individuals were tested for immunoglobulin A [IgA], IgG and IgM as well as complement factors C3 and C4 by single radial immunodiffusion assay. Determination of serum sialic acid for control, patients and calibration samples was performed by the resorcinol method.

RESULTS:

The levels of all three classes of immunoglobulin and complement factors were higher in typhoid and brucellosis patients than in healthy individuals, beside significant increases in the serum levels of SA in patients with typhoid fever and brucellosis. There was no significant difference between serum levels of SA in typhoid and brucellosis patients as compared to the control group of healthy individuals. However, we were not able to observe a clinically meaningful difference with respect to sialic acid levels between these two categories.

CONCLUSION:

Sialic acid is a general disease marker than a specific marker and the none specificity of increase makes SA determination unsuitable as a specific marker. However it may have a clinical utility, which can be used in conjunction with other test.

KEY WORD: Brucellosis , Complement Factor, Immunoglobulin ,Sialic Acid, Typhoid Fever .

INTRODUCTION:

Sialic acid (called neuraminic acid) is the designation given to a family of over 40 naturally occurring nine-carbon keto sugars acids derived from the parent compound 2-keto- 3-deoxy-5-acetamido-D-glycero-D-galacto-nonulosonic acid (Nacetylneuraminic acid [Neu5Ac]). The sialic acids and related nonulosonates are unique in nature by representing the only nine-carbon sugars found in prokaryotes.

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In eukaryotes, sialic acids have evolved to mediate a diverse range of cell-cell and cell-molecule interactions, including (i) stabilizing glycoconjugates and cell membranes due to charge-charge repulsion, (ii) mediating cell-cell regulation and acting as chemical messengers, (iii) regulating transmembrane receptor function, (iv) affecting membrane transport, (v) controlling the half-lives of circulating glycoproteins and cells, and (vi) contributing to the permselectivity of the glomerular endothelium and slit diaphragm [(1)]. Some bacteria have evolved a de novo pathway for sialic acid biosynthesis that differs from the eukaryotic method, whereas other microbes use truncated synthetic pathways utilizing sialyl

precursors scavenged from animal hosts [2]. Sialic acid moieties are also ligands for a diverse array of exogenous lectins, most notably those on invasive pathogens, where binding to host sialyl-determinants is prerequisite to pathogenesis [3, 4]. Microbial sialic acid metabolism has now been firmly established as a virulence determinant in a range of infectious diseases [5]. The summer problem in Iraq, food poisoning fever, is usually acquired through ingestion of water, milk or food contaminated by many pathogenic bacteria such us *Salmonella spp*. and *Brucella spp*..These Gram –negative bacteria induces a disease in human causes typhoid fever and brucellosis. Typhoid fever and brucellosis are frequent causes of bloodstream infections in many countries [6, 7]. The diagnosis of these infections is challenging because they can have diverse clinical manifestations with symptoms that overlap with a wide spectrum of other diseases [8]. Cultures of blood or bone marrow are the most definitive diagnostic methods. In many developing countries, both diseases are diagnosed on clinical grounds and treated empirically [9, 10]. Serological assays are often utilized as a diagnostic tool (8, 16); however, neither Widal nor Rose-Bengal agglutination assays are sufficiently sensitive, specific, or practical in areas of endemicity [11, 12]. In this study, it was undertaken to determine the sensitivity and specificity of clinical application of sialic acid in the evaluation of humoral immune response in patients with typhoid fever and brucellosis. Furthermore , the role of sialic acid (SA) was investigated as a possible biological marker and to assist diagnosis of disease.

MATERIALS AND METHODS:

Patients:

This study consisted of patients treated for typhoid fever and brucellosis according to complete physical examination and final diagnosis by Widal test and Rose-Bengal test besides the healthy controls.

The typhoid fever patients (n=35, median age 35 years, range 20-50) and brucellosis patients (n=35, median age 30 years, range 15-45) were referred to different hospital in Baghdad. The control group (n=60, median age 37 years, range 24-50) had no evidence of any type of fever. The blood samples were allowed to coagulate at room temperature and centrifuged at 3000 rpm for 10 min. The sera were separated and stored at -20 ^{0}C.

Determination of Sialic Acid:

Determination of serum sialic acid for control, patients and calibration samples was performed by the resorcinol method by Svenerholm 1958 (13).

Twenty µL for each concentration (10, 20, 30, 40) µg ml^{-1} of calibration samples or serum samples was putt in clean and sterile test tube, 980 µL distilled water was added for each tube, the solution mixed and putt in an ice bath. One ml of resorcinol reagent (coloring S A) was added to each tube.

The tubes were putt in water bath at 100 ^{0}C for 15 min, and then transferred to ice bath for 10 min, 2 ml of (Butyl acetate / Methanol) solution was added with a good mixing. The samples centrifuged at 3000 rpm for 10 min. Reading the absorbency was curried out at wavelength 580 nm.

Detection of Immunoglobulins and Complement Factors: Serum levels of IgA, , IgG , IgM , C3 and C4 mg dl^{-1} were estimated using the single radial immunodiffusion method (14).

The method is based on measuring the diameter of the precipitation ring and the immunoglobulins and complement factors level was obtained from the table accompanying the test kit provided by Biomeghreb (Tunisia).

Statistical Data Analysis:

Data were statistically analyzed using SPSS statistical software (version 11.5) by analysis of variance (ANOVA) test. The values are given as mean ± standard error.

RESULTS:

1. SA levels in fever patients.

A significant increase (P< 0. 01) was observed in the serum levels of SA in typhoid fever patients (92.24±4.77 mg dl^{-1}) and brucellosis patients (103.0.5±23.27 mg dl^{-1}) as compared to the control group of healthy individuals (64.82±23.27 mg dl^{-1}). Sialic acid levels in both fever patients groups did not show any statistically significant difference (p> 0.05) (Figure 1).Table 1 shows that the serum sialic acid has low specificity 29% in typhoid fever group and 23% in brucellosis group in spite of mild high in sensitivity . However, in the group of typhoid fever the positive results were 24 of 35 while in brucellosis the positive results were 27 of 35 (Table 1).

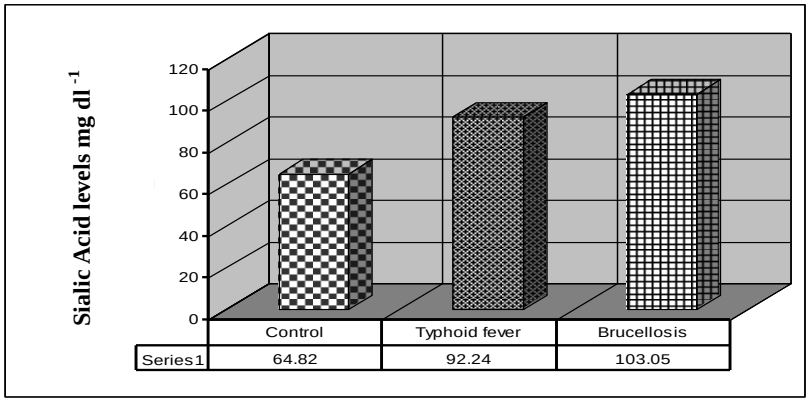

Figure 1: Serum sialic acid levels in patients (mg dl $^{-1}$).

Table1: Sensitivity and specificity of serum sialic acid in typhoid fever and brucellosis.

Patients group	Serum total sialic acid result (Number of patients)		Sensitivity* (%)	Specificity** (%)
	Positive results	Negative results		
Typhoid fever group	25	10	71	29
Brucellosis group	27	8	77	23

***** Calculated by dividing the number of positive results by the total number of patients (Positive results and negative results)

****** Calculated by dividing the number of negative results by the total number of patients (Positive results and negative results)

2. Immunoglobulin levels in fever patients.

- IgA levels in fever patients. A significant elevation ($p<0.05$) of IgA was observed in typhoid fever patients (328.73±37.99 mg dl^{-1}) and brucellosis patients (287.40±38.40 mg dl^{-1}) as compared to the control group of healthy individuals (154.20±23.40 mg dl^{-1}) (Figure 2).

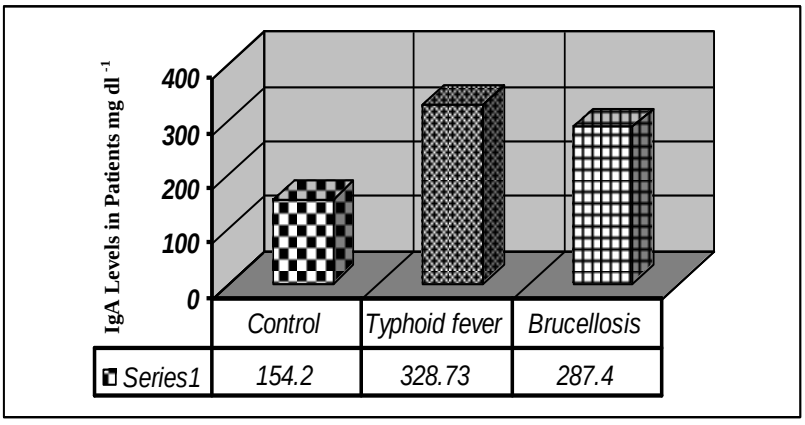

Figure 2: Serum IgA levels in patients (mg dl $^{-1}$).

- **IgG levels in fever patients.** A highly significant elevation ($p<0.001$) of IgG was observed in typhoid fever patients (1227.84±38.98 mg dl^{-1}) and brucellosis patients (1132.16±29.01 mg dl^{-1}) as compared to the control group of healthy individuals (823.55±22.45 mg dl^{-1}) (Figure 3).

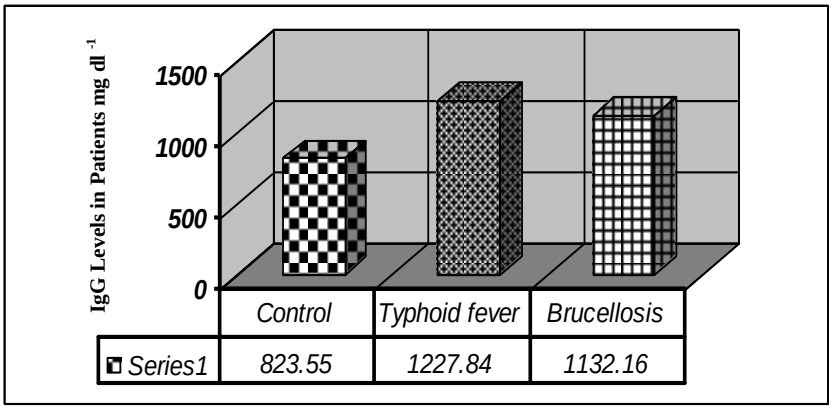

Figure 3: Serum IgG levels in patients (mg dl^{-1}).

- **IgM levels in fever patients.** A highly significant elevation ($p<0.001$) of IgM was observed in typhoid fever patients (217.46±11.68 mg dl^{-1}) and brucellosis patients (208.65±18.15 mg dl^{-1}) as compared to the control group of healthy individuals (83.55±13.15 mg dl^{-1}) (Figure 4).

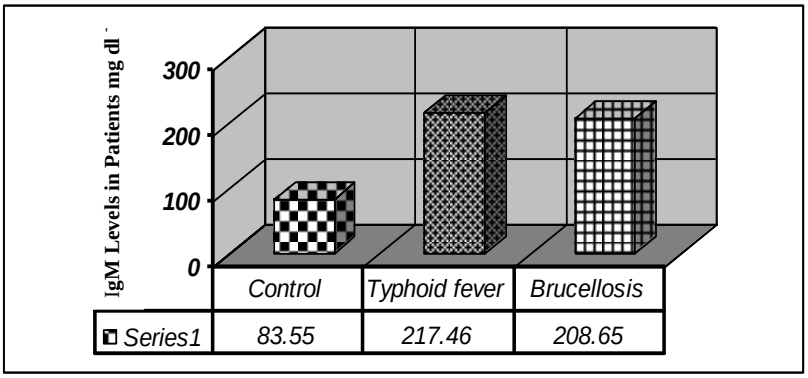

Figure 4: Serum IgM levels in patients (mg dl^{-1}).

3. Complement factors levels in fever patients. A significant increase ($P< 0.05$) was observed in the serum levels of complement factors C3 and C4 in typhoid fever patients (105.86±37.33; 55.93±14.32 mg dl^{-1}; respectively) and brucellosis patients (112.40±30.80; 51.90±2.90 mg dl^{-1}; respectively) as compared to the control group of healthy individuals (33.45±2.24; 29.56±0.75 mg dl^{-1}; respectively) (Figure 5).

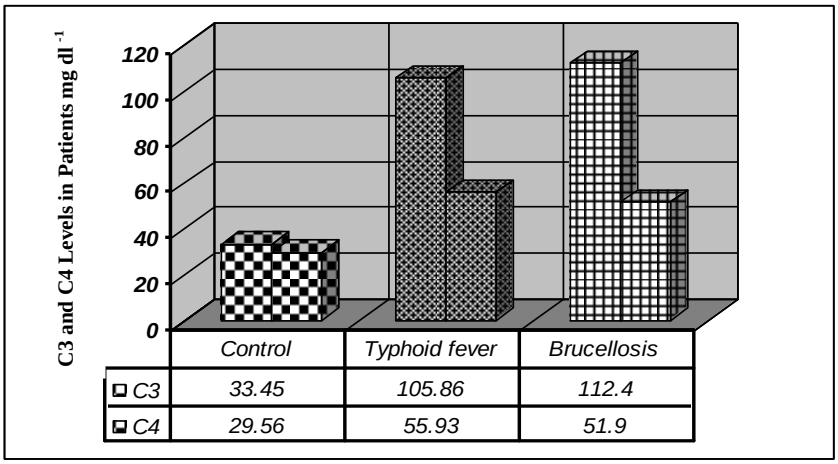

Figure 5: Serum C3 and C4 levels in patients (mg dl $^{-1}$).

DISSCUSION:

Sialic acids are not only the most interesting molecules in the world, but also the most important (15). Interestingly, this study showed there is a significant difference in the levels of serum sialic acid in patients with typhoid fever and brucellosis patients as compared to the healthy individuals. Increased concentration of sialic acid in various tissues and fluids has been observed which may be due at least in part to defective de novo synthesis, transport, storage, catabolism, excretion and / or metabolic regulation of sialic acid in the cells or may be increased through changes in the biosynthesis and post translational glycosylation processing of acute-protein phase glycoprotein in the liver (16). Or the increase in SA shedding may primarily reflect an increase in the activity of microbial sialidase. As these Gram-negative bacteria contain endotoxin (lipopolysaccharide [LPS]), which is a very potent immune stimulator, it was likely that the strong immune responses obtained were further strengthened by the presence of LPS (5). Although, as discussed above, the systemic concentration of free sialic acid is low, localized increases caused by inflammatory cues could create foci of highly concentrated free sialic acids independently of microbial sialidase action. This suggestion follows from observations that inflammatory neutrophils undergo an interleukin-8-inducible recruitment of intracellular sialidase to the cell surface, where release of bound sialic acids from surface molecules and the surfaces of cells in the surrounding environment has the potential to raise local sialic acid levels (17). Thus, inflammation triggered by microbial products such as LPS may trigger events resulting in increased free sialic acids during an infection. Cell wall components or surface structures such as LPS, and protein antigens [flagellin] can induce the expression of proinflammatory cytokines (TNF-α, IL-1, IL-6, IL-12, and IL-18) via signaling through Toll-like receptors (type I transmembrane receptor) these cytokines and chemokines recruit cells of the immune system (18). The present study has also revealed increases in the levels of the immunoglobulins (IgA, IgG and IgM) and complement factors (C3 and C4) in the sera of typhoid fever and brucellosis patients. This increase seems to have a relationship with the elevated levels of SA in the sera of these patients. The significant increase in serum immunoglobulins of typhoid fever and brucellosis patients in the present study is consistent with what was demonstrated by previous studies (19,20). These antibodies possess particularly valuable features for fighting intracellular pathogens such as *Salmonella* (21). In human brucellosis, specific immunoglobulin M (IgM) antibodies usually develop early in the infection and remain present for several weeks to months (22, 23). Specific IgG antibodies tend to develop somewhat later but may remain present, albeit at low levels, for months to years also after the patient has recovered (24, 25, 26). Consistent with the fact that the complement level rises in most inflammatory conditions (27, 28), a very marked rise in C3 and C4 serum level was noted in typhoid fever and brucellosis patients.

The innate immune system plays an essential role in the early response to pathogenic bacteria and may be enough to control progression to disease in most subclinical infections. Also, complement fixation on the bacterial surface could promote complement-receptor-facilitated uptake by phagocytes and activation of the complement cascade via the classical and alternative pathways plays a major role in resistance to many Gram-negative bacteria (29). When bacteria invades the body it initiates an active cell mediated immune response, which is orchestrated by activated macrophages aided by T helper cells; and the battle is intensified by a humoral B cell response. However, since antibodies, cytokines and complement factors are glycoproteins in nature, the elevation in their levels can be directly explained by the fact that the levels of serum glycoproteins are elevated in typhoid fever and brucellosis patients. In addition, the immune functions that are affected by the changes in the sialic acid content on cell surface of immune and host cells include self/non-self discrimination, production of natural antibodies to desialylated host cells , complement activation, macrophage-mediated phagocytosis lymphocyte-mediated cytotoxicity , natural killer cells , immune cell adhesion , antigen-specific interactions , and several other important immune functions (30,31). The development of cell-mediated immune response (CMI) in typhoid fever and brucellosis with correlate in serum sialic acid levels has not been studied before of our knowledge .Thus, we attempted in this study to more precisely define the potential role of sialic acid as a diagnostic marker in febrile fever by comparing its levels in typhoid fever and brucellosis. However, we were not able to observe a clinically meaningful difference with respect to sialic acid levels between these two categories. That sialic acid is a general disease marker than a specific marker and the none specificity of increase makes SA determination unsuitable as a specific marker and also our result goes with finding reported by Isitmangil et al. (32) that although high SA in serum but it does not have any clinical significant nor is it important as a diagnostic marker. Thus the association between serum SA and infectious disease may reflect the role of mechanism other than the severity of disease.

CONCLUSION: In general it could be concluded that serum sialic acid, has limited usefulness as an aid in the diagnosis of disease. However it may have clinical utility, which can be used in conjunction with other test.

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