

EVALUATION OF SERUM TOTAL, LIPID AND PROTEIN ASSOCIATED SIALIC ACIDS LEVELS AS AN INFLAMMATORY MARKERS IN TYPHOID FEVER PATIENTS

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Abstract

Total sialic acid (TSA) and its fraction lipid and protein associated sialic acids (LASA and PASA) were measured in serum belonged to 100 patients with typhoid fever, and 50 normal subjects considered as normal healthy controls. Data analysis shows that the levels of TSA (91.58 ± 22.15 mg/dL), LASA (28.88 ± 7.28 mg/dL) and PASA (60.87 ± 15.50 mg/dL) were significantly ($P < 0.0001$) higher in serum typhoid fever patients compared with normal controls (66.0 ± 20.0 mg/dL), (16.0 ± 4.0 mg/dL) and (42.0 ± 7.0 mg/dL) respectively, but there is no statistically significant differences seen between females and males patient groups in regard to TSA, LASA and PASA levels.

The results showed the levels of TSA and its fraction (LASA and PASA) in typhoid fever patients are higher than normal which showed normal levels, further study is required to explore the binding of sialic acid to the specific site of proteins and lipids to consider these inflammatory markers in monitoring and progression for typhoid fever disease, and evaluating the effectiveness of various therapeutic approaches.

Keywords: Inflammatory marker, Sialic acid, Typhoid fever.

Introduction

Inflammatory markers can be measured in serum, plasma or other body fluids, their concentrations changed in many diseases markedly in typhoid fever⁽¹⁾.

Typhoid fever is an acute illness associated with fever caused by the *Salmonella* Typhi bacteria^(2,3). The bacteria multiplies in the gallbladder, bile ducts, or liver and passes in to the bowel⁽⁴⁾. These chronic carries may have no symptoms and can be the source of new out breaks of typhoid fever for many years⁽⁵⁾. N-acetylneuraminic (NANA) is the most prominent sialic acid (SA) in eukaryotes, it plays a central role in the biochemical functioning of humans⁽⁶⁾. It is negatively charged nine-carbon keto sugar, commonly attached by an glycosidic linkage to the non-reducing residue of the carbohydrate to chains of glycoproteins and glycolipids⁽⁷⁾. Sialic acid participates in the stabilization of the conformation of glycoproteins, glycolipids various mucoproteins and cellular membranes^(8,9).

Lipid associated sialic acid (LASA) and protein associated sialic acid (PASA) are alkylated derivatives of neuraminic acid, the carbohydrate moiety characterized the cohesive, adhesive and antigenic properties by its effect on cell-to-cell contacts^(10,11). The structural diversity of sialic acid is exploited by viruses, bacteria, toxins and antibodies⁽¹²⁾.

Little is known about the utility of sialic acid as a marker for inflammatory process. Recent study was found that the concentration of total sialic acid (TSA) in the human serum is higher in a number of pathological states where the indulging pathology is either of tissue destruction, tissue profilation, depolymerization and inflammation⁽¹³⁾. An elevation of serum total sialic acid concentration has been reported in cancer⁽¹⁴⁾, type 2 diabetics renal failure⁽¹⁵⁾. Liver cirrhosis^(16, 17), cardiovascular disease⁽¹⁸⁾ and infection⁽¹⁹⁾.

The results of these investigations are conflicting, particularly the concerning of the total sialic acid content of glycoproteins, and

glycolipid levels. Other findings have stimulated interest in measuring serum sialoglycoproteins and sialoglycolipids as a possible marker to detect the severity of typhoid fever as inflammatory disease⁽²⁰⁾. The mechanism of the elevation of total sialic acid in serum belongs patients with typhoid fever is very complex and can lead to variable total (SA) results in different types of inflammatory diseases⁽¹³⁾.

The objective of the present investigation is to evaluate the relative usefulness of the determination of serum levels of (TSA), (LASA), and (PASA) in diagnosis and prognosis of typhoid disease, and for detecting and monitoring the progression of the disease in a large population of patients.

Materials and Methods

All chemicals and reagents used in this study were of analar grade unless otherwise specified, and were obtained from Fluka (UK), Hopkins and William Sigma chemical (NANA) and Riedel-Deherform companies.

In a period of eight months, one hundred (100) typhoid fever patients (63 females and 37 males) of age range (10-80 years), both females and males belongs different socioeconomic classes were selected from Al-Saddir hospital in Baghdad, patients were subjected to clinical examination to confirm the diagnosis of typhoid disease, which was based on clinical features, Widal test and blood culture⁽²¹⁾. Widal test of quite sensitive that can be a diagnostic value with high specificity and sensitivity.

Fifty (50) normal controls (28 females and 22 males) of age range (13-70 years) with socioeconomically matching were taken having no history of typhoid disease.

Whole blood was drawn from patients and normal controls. The blood was allowed to coagulate at room temperature, and was centrifuged at 2000 xg for 15 minutes. The resulting sera were placed in test tubes and stored frozen at (-20 °C) until used.

Stability studies showed that TSA and its derivatives were constant under these conditions for up to 6 months⁽¹⁴⁾.

Determination of total sialic acid

Distilled and deionised water was used in this study. The assay was done according to the resorcinol method cited in (Lindberg 1997) with modification⁽²²⁾. The principle of this colorimetric method depends on the formation of chromogen by addition of resorsinol reagent. The chromogen was extracted by butylacetate / methanol solvent (85-15 v/v) then the concentrations were measured at 630nm.

Standard curve of total sialic acid was prepared by plotting absorbance versus (TSA) in microgram (Fig-1), then this concentration was converting to milligram in 100 ml of serum using the formula:

$$\text{Concentration of the test} = \frac{\text{Absorbance}}{0.0188} \times 5 = \text{mg / dL}$$

Determination of Lipid associated sialic acid

Serum LASA values were determined according to (Katapoidis 1980)⁽²³⁾. Sialolipids were extracted from serum by cold chloroform/ methanol solution for 30 seconds. After centrifugation, the upper layer (presents sialolipid fraction) precipitated by phosphotungstic acid, the precipitant was dissolved by distilled- deionized water. Then resorcinol- HCl reagent was added to form chromogen which then extracted to read as in TSA assay⁽²²⁾.

Serum PASA values were determined by precipitating the glycoprotein by ethanol solution and then dissolved in 0.1 N NaOH. The chromogen was developed as in TSA assay⁽²²⁾.

Statistical analysis

All data are presented as(mean ± SD). The results were analyzed according to the available statistical package of Social Sciences Version 15.0 (SPSS- 150). The comparison within and among group were done using one way ANOVA test, using student t-test (two paired and two tailed), and taking P-value ≤ 0.05 as the lowest limit of significance.

Results

The demographic characteristics of this study is shown in Tables (1) (2) and (3). Table (1) presents the mean \pm SD of total sialic acid (TSA) expressed as mg/dL in serum of typhoid patients and normal controls with their sex, biostatistical calculation and t-test. Serum TSA from the typhoid patients shows a significant increase (91.58 ± 22.15) mg/dL when compared to the normal control (66.0 ± 20.0) mg/dL ($P < 0.0001$) Fig. (2).

No significant differences in TSA values were observed between females and males patients.

Table (2) and Fig.(3) show a higher significant values of serum LASA among typhoid fever patients (28.88 ± 7.28) mg/ dL as compared to the normal controls (16.0 ± 4.0) mg/dL ($P < 0.0001$), but no significant differences were noted when the LASA values for the females patients were compared to the males.

Table (3) Fig. (4) compare the serum PASA values of typhoid patients to normal controls, a significant increase ($P < 0.0001$) in PASA values was seen in typhoid patients (60.87 ± 15.50) mg/dL as compared to normal control subjects (42.0 ± 7.0) mg/d L.

No significant differences were observed for PASA values between females and males patients.

Discussion

The results reported in this study highlighted several observations, some of them documented the previous publications and others added more informations about study.

Sialic acid is usually bound to glycoproteins, glycolipids, oligosaccharides, polysaccharides and a small amount is free in the body⁽²⁴⁾. The most common sialic acid is N-acetylneuraminic acid (NANA) and N- neuraminic acid (Ne U5 GC).⁽²⁵⁾ Biochemical alteration in total sialic acids concentrations has been considerable interest as a potential inflammatory marker for several diseases^(26,27,28). Previous reports have indicated that serum sialic acids levels were associated with an increase risk of acute myocardial infraction^(29,13), inflammatory

disease^(30,24,19), malignancy^(31,26), diabetes mellitus⁽¹⁵⁾, diabetic nephropathy⁽³²⁾ and bacterial infections^(33,34).

This study was carried out to evaluate the levels of (TSA), (LASA) and (PASA) in patients with typhoid fever and normal controls and to determine which of these markers might be most useful for detecting and monitoring the progression of typhoid disease. Serum TSA levels were significant increased in the patients when compared to normal controls ($P < 0.0001$), the actual cause of this elevation is not known, however several researchers have proposed a variety of mechanisms, one such mechanism includes the shedding of sialic acid into the circulation as a result of cell membrane damage, tissue proliferation, massive tissue destruction and inflammation. These results were nearly consistent with those of previous research workers, depicting of marked increase in serum sialic acid concentration following the inflammatory injury process⁽²⁸⁾ and rheumatoid arthritis⁽³⁵⁾.

In the present investigation, LASA and PASA levels were significantly higher in serum of typhoid fever patients ($P < 0.0001$) as compared to normal, this marked increased in LASA and PASA levels may indicate the deformation has been occurred in the cells, and an amount of sialic acid containing glycolipid and/ or glycoprotein, (mainly acute phase proteins, such as alpha acid glycoprotein and alpha antitrypsin), were released from vascular cells into blood stream⁽³⁶⁾. Previous observations suggested that inflammatory neutrophils undergo a interleukin-8- inducible recruitment of intracellular sialidase (s) to the cell surface, where the release of bound sialic acid from surface molecules and the surfaces of cells in the surrounding environment has the potential to raise local sialic acid concentration, thus inflammation triggered by microbial products such as endotoxin lipopolysaccharide (LPS) may triggered events resulting in increase the sialic acid during infection⁽³⁷⁾.

The results suggesting that these markers may be useful indicators in diagnosis and prognosis in inflammatory typhoid disease.

Table (1)
Serum total sialic acid levels (mg/d L) in controls and typhoid patients with their sex, biostatistical calculations and standard t-test.

TSA (mg/d L)	Control	Patients	Patients	
			Males	Females
Sample size	50	100	37	63
Mean \pm SD	66.0 \pm 20.0	91.58 \pm 22.15	88.95 \pm 21.40	93.13 \pm 22.61
Range	45- 70	50 - 150	50 – 142	56 – 150
Standard error of mean	2.8	2.22	3.52	2.85
Confidence interval of mean	60.4 - 71.6	87.24 – 95.92	82.05 – 95.84	87.54 - 98.71
t – test	6.881		0.910	
Probability	0.0001 (Highly significant)		0.365 Not significant	

Table (2)
Serum Lipid-associated sialic acid (LASA) values (mg/dL) in controls and typhoid patients with their sex, biostatistical calculations and standard t-test.

LASA(mg/d L)	Control	Patients	Patients	
			Males	Females
Sample size	50	100	37	63
Mean \pm SD	16.0 \pm 4.0	28.88 \pm 7.28	26.65 \pm 6.57	30.19 \pm 7.41
Range	6 - 24	15 - 48	18 – 47	15 – 48
Standard error of mean	0.56	0.73	1.08	0.93
Confidence interval of mean	17.13 – 14.87	27.45 – 30.31	24.53 - 28.76	28.36 - 32.02
t – test	11.64		2.404	
Probability	0.0001 (Highly significant)		0.018 Notsignificant	

Table (3)
Serum protein -associated sialic acid (PASA) values (mg/dL) in controls and typhoid patients with their sex, biostatistical calculations and standard t-test.

PASA(mg/d L)	Control	Patients	Patients	
			Males	Females
Sample size	50	100	37	63
Mean \pm SD	42.0 \pm 7.0	60.87 \pm 15.50	60.5 \pm 15.0	61.08 \pm 15.87
Range	28 - 53	33 - 110	36 – 93	33 – 110
Standard error of mean	1.00	1.55	2.40	2.00
Confidence interval of mean	44.0 – 40.0	57.83 – 63.91	56.1 – 65.9	57.16 – 65.00
t – test	8.19		0.175	
Probability	0.0001 (Highly significant)		0. 861 Not significant	

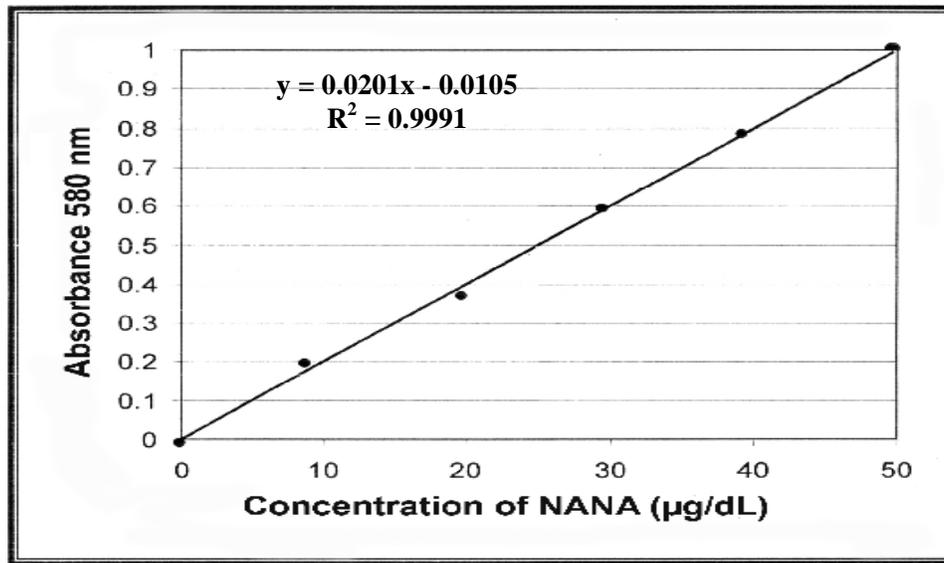


Fig.(1) : Standard curve for determination of sialic acid by plotting absorbance versus NANA (microgram).

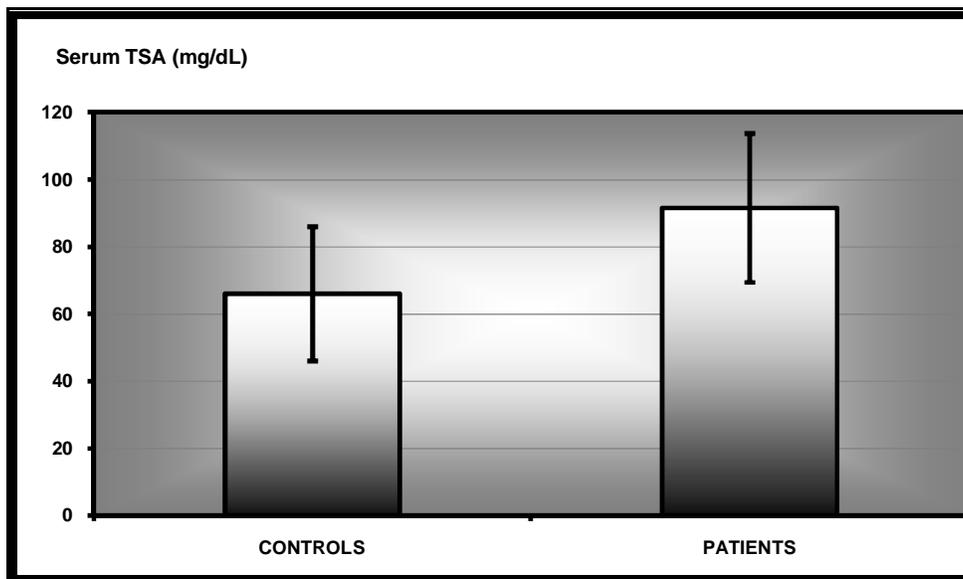


Fig.(2) : Serum TSA in the sera of typhoid fever subjects and the control group.

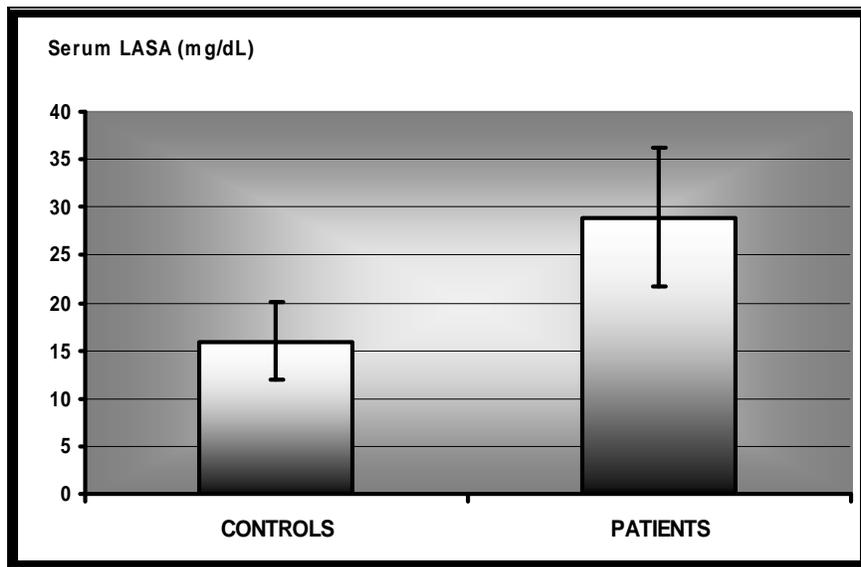


Fig. (3) : Serum LASA in the sera of typhoid fever subjects and the control group.

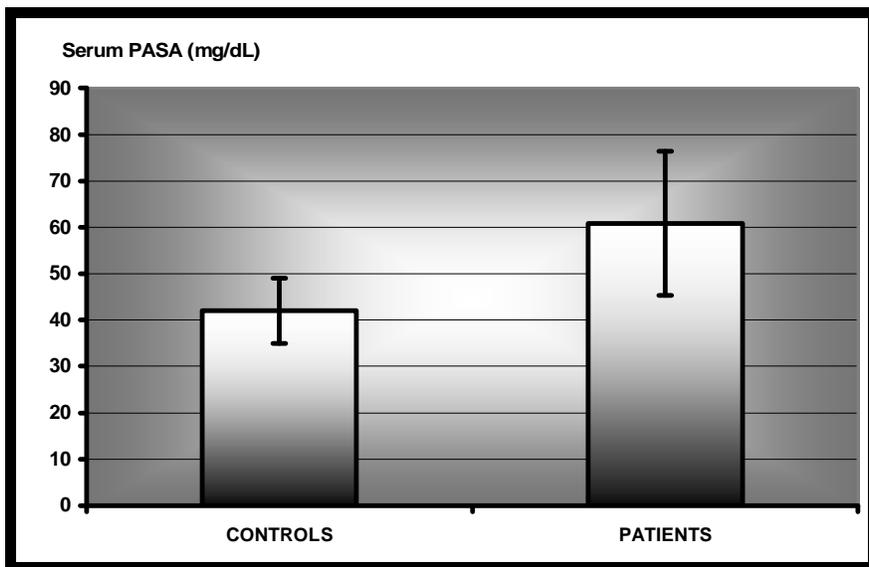


Fig. (4) : Serum PASA in the sera of typhoid patients and the control group.

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الخلاصة

تم قياس تغيرات تركيز حمض السيلاليك الكلي (TSA)، حمض السيلاليك الدهني (LASA) والبروتيني (PASA) في مصل الدم لبيان الأهمية السريرية كعامل خطورة عند 100 شخص مصابين بحمى التيفوئيد، إضافة إلى (50) شخص سوي لغرض المقارنة (كعامل تحكم سوي). أظهر التحليل الاحصائي للنتائج وجود زيادة دالة في مستوى حمض السيلاليك الكلي (91.58 ± 22.15 mg/dL) و تركيز حمض السيلاليك الدهني (28.88±7.28 mg/dL) وكذلك حمض السيلاليك البروتيني (60.87 ± 15.50 mg/dL) (P<0.0001). في مصل دم المرضى المصابين عند مقارنتها مع نتائج نماذج مجموعة التحكم السوية (66.0±20.0mg/dL) (16.0±4.0mg/dL) (42.0±7.0mg/dL) على التوالي، بينما لا يوجد اختلاف احصائي واضح في مستويات (TSA, LASA, PASA) في مصل دم الاناث المصابات بحمى التيفوئيد من جهة وبين الذكور المصابين من جهة أخرى.

يستنتج بأن مستويات حمض السيلاليك الكلي (TSA) واجزاءه (LASA, PASA) في مرضى حمى التيفوئيد هي أعلى من تلك في الأشخاص الاصحاء الذين أظهروا مستويات طبيعية. وهذه النتائج تتطلب دراسة إضافية لمعرفة كيفية ارتباط حمض السيلاليك بالبروتينات والدهون لكي تتأكد اعتماد هذه الواسمات الالتهابية في تقدير مدى تطور مرض حمى التيفوئيد ومعرفة فعالية الطرق المختلفة المتبعة في العلاج.