

Total Serum Sialic Acid Among Enteric Fever Patients

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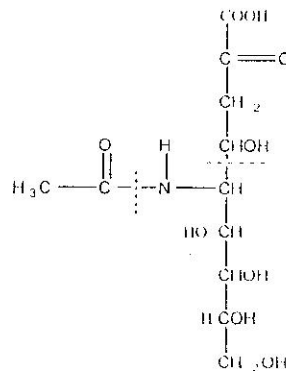
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Summary

In this study, serum total sialic acid concentration were tested as a serological marker of diseases activity, to evaluate the rules of the test in the diagnosis of enteric fever, (TSA) was measured in the serum of (50) patients with typhi fever, (50) patients with paratyphi fever, (50) patients with disease other then enteric fever as pathological control, (50) normal healthy persons as normal healthy control. This study revealed that TSA increases significantly in typhoid patients and pathological control (88 mg/dl) & (99 mg/dl) respectively as compared to normal healthy, but there is no significant differences between thyphoid patients as compare to pathological control ($P > 0.05$), and there is no difference between typhi fever and paratyphi fever ($P > 0.05$). However the test has low specificity and sensitivity leading to sever limitation of the test, which seems of no value as biochemical marker in typhoid patients, so that we can say that serum (TSA) is a general marker for fever rather than a specific marker.

Introduction

The sialic acids (SAs) refer to a family of compounds derived from an substituent nine- carbon chain called neuraminic acid. (nine carbon polyhydroxy keto acid) $C_9H_{17}O_8N$. The N-acetyl, N-glycolyl, N-O diacetyl (neuraminic acid) are collectively termed Sialic acid Robert et al., 1993. The most common form of SA is N-acetylc neuraminic acid (NANA).



Sialic acid composed a major of negative charge on the surface of animal and mammalian Cells and are important in regulating intercellular conlins and

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inter action of charged macromolecules with the cell surface. Trace amount of SA are found free in tissue and secretion most are bound carbohydrate moiety of glycoconjugate and glycolipids. They occupied the terminal site in glycoconjugate where they can be released by either mild acid or enzymatic hydrolysis. (Ibrahim & Al-Nassir, 1992). In other hand Robert, (1993) refers to that, sialic acid may present in human body in three forms:

- 1- Free sialic acid present in trace amount in serum.
- 2- Protein associated sialic acid as a terminal sugar of glycoprotein in the cell membrane and other cell component.
- 3- Lipid associated sialic acid, which is present in glycolipid.

Studies of some disease especially cancer, collagen diseases and inflammatory diseases, have indicated relationship between the level of sialic in serum and the nature of disease (Salomone, et al., 1998). The high concentration of SA in the serum with bacteria infection could be related to sialoglycoprotein from the liver or could be as and mucoprotein in inflammatory processes (Bircan et al., 1998). Goldsby et al., (2000) reported in his study, the membrane of most mammalian cells have high levels of sialic acid, which contributed to the rapid inactivation of bound C3b molecules on host cell. Consequently this binding rarely leads to further reactions on the host cell membrane. Because many foreign antigenic surface eg. Bacterial cells walls, have only low levels of sialic acid C3b bound to these surface remains active for a longer time. The present study was undertaken to determine the potential clinical application of (SA) in the evaluation of patients with enteric fever (which is caused by *Salmonella typhi* and *paratyphi A* and *B*, . Further more assess the possible ability of this biomarker, in the term of sever-

ity of the enteric fever and to assist diagnosis of disease.

Materials And Methods

Subjects : - four groups of subjects were studied whom were attended to different hospitals or the period from July to December 2000. The first group (Group 1) involved 50 patients (27 females & 23 males) with signs and symptoms of typhoid fever according to complete physical examination and final diagnosis by widal test. The ages of these patients varied between 30-33 years. The second group (Group 2) involved 50 patients (23 female & 27 male) with paratyphi fever, those patients were with age range between (27-30 years). The fourth group involved 50 patients (22 females & 28 males) with fever other than enteric fever like (urinary tract infection, pneumonia, gastroenteritis, measles, mumps, rheumatic fever, hepatitis, tonsillitis and glomerulonephritis) according to physical examination and questioner, and those patients with age range between (30-32 years). This group considered as pathological control. The fourth group involved 50 healthy persons (25 females & 25 males) with age ranges between (30-33) years were considered as normal control.

Blood Sampling: The blood samples were allowed to coagulate at room temperature and centrifuged at 3000 r.p.m for 20 min. the residual sera were separated and stored at 20 °C.

Total serum sialic acid determination (TSA)

The principle of this method depends on the formation of chromagen in addition to resorcinol reagent into the test tube.

The chromogen formed was extracted by butyle acetate methanol reagent, and measured at 580.

Reagents: – Reagents (resorcinol stock) : made up by dissolving 2 gms of resorcinol in 100 ml of distal water. The reagent is stable for many months in the refrigerator.

Reagent (2) (resorcinol -- HCl) : 10 ml of stock solution is added to a mixture of 80 ml of concentration HCl and 0.25 ml of 0.1 M CuSO₄.

The volume is then made up 100 ml with water, this should be prepared at least four hours before use and stable for two weeks in the refrigerator.

Reagent (3): butyl acetate / methanol:- 85 ml of butyl acetate is added to 15 ml of methanol.

Procedure: – The reactions was performed in 8 50 in pyrex test tube labeled as test. Standard and blank into which the following reagents were pipetted as following in table (1).

Table (1): The amount of reagents added in determination of SA.

Reagent	Test	Standard	Blank
Sample	20 µl		
D water	980 µl	980 µl	1 ml
Standard		20 µl	
Reagent 2	1 ml	1 ml	1 ml
Reagent 3	2 ml	2 ml	2 ml

The tubes were capped with glass tubes and heated for 15 min. in a boiling water bath (100 °C) and then cooled to tap water or ice bath for 10 min. then centrifuged for 10 min at 3000 rpm, the extrated chromophore was read at 580 nm.

Calculation: – Standard curve composed of microgram of NANA prepared by plotting absorbance at 580 nm versus NANA. (microgram) as shown in figure (1).

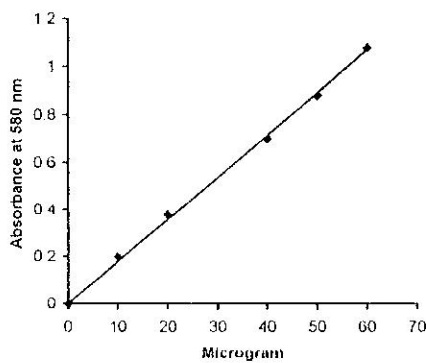


Figure (1) : Calibration curve for the determination of silicic acid by plotting absorbance versus NANA (microgram).

*concentration of the test =
$$\frac{\text{Absorbance}}{0.0188} \times 5 = \text{mg / dl}$$

* the concentration of the test calculated by using this equation after the calculation of the Linear slop of the curve and convert microgram to milligram in hundred ml of serum.

Statistical Analysis

Student t-test was used to determined whether the mean value for the serum (TSA) is significantly different in the normal healthy control, pathological control and the enteric fever patients.

P-value < 0.05 were consider significant.

Results and discussion

Table (2): The mean concentration of TSA mg / dl expressed as mg / dl in sera of all groups.

Serum TSA mg/dl	Normal healthy	Pathological control	Group 1	Group 2
Sample size	50	50	50	50
Mean concentration of silicic acid	60	90	88	90
Range	53-78	61-112	75-117	55-122
Standard error	1.0	0.9	2.5	1.5
Confidence interval	61-68	88-92	81-92	87-91
t-test		22*	8-1*	11-3*
			0.9**	1.6**
Probable p-value		P < 0.05	P	P < 0.05*
			0.65*	NS**
			NS**	NS**

* t-test and p-value for pathological control, group 1 and 2 typhoid fever patients s ompared o ormal ealthy controls.

** t-test and p-value of group 1 and 2 typhi patients as compared to pathological controls.

** t-test and p-value for group 1 typhi patients as compared to groups 2 paratyphi patients.

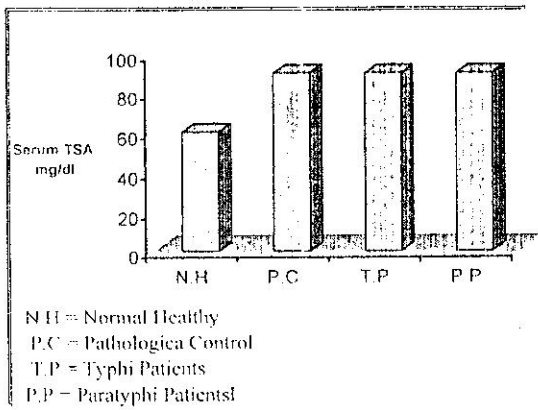


Figure (2): Serum TSA level in normal healthy, pathological controls, typhi patient and paratyphi patients.

Table (3): The predictive value for the serum TSA in typhoid fever.

	Sensitivity	Specificity	Positive predictive	Negative productivity	Efficiency
Typhi group	76%	30%	52%	55%	53%
Paratyphi group	70%	30%	50%	50%	50%

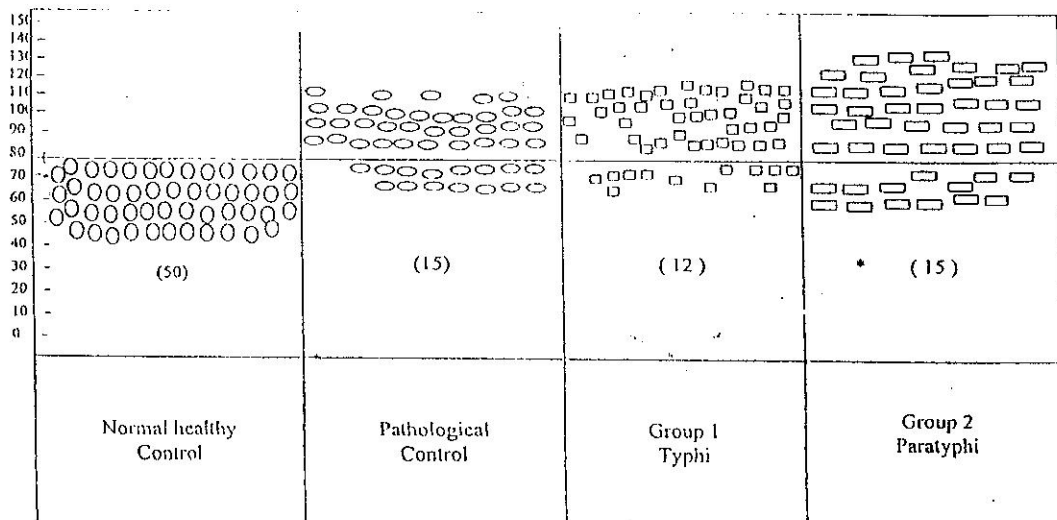


Figure (3) Distribution of (TSA) level sera of normal , pathological controls group 1 and 2 typhi patient (mean \pm SD). Horizontal line indicated the cut off value.

Result And discussion

Table (2) and figure (2) shows the mean concentration of TSA expressed as mg/dl in sera of Typhi patients (group 1) and paratyphi patients (group 2) pathological controls and normal healthy, from which there were significant increased for serum (TSA) in pathological control. Group 1 and group 2 typhoid when compared to normal healthy controls ($P > 0.05$).

Also there is no significant difference in serum (TSA) level between pathological control as compared to both groups of typhoid patients and paratyphoid, furthermore there is no significant differences between typhi patient (group 1) and paratyphi patient (group 2).

Figure (3) illustrates the distribution of TSA level in sera of normal healthy, pathological control and typhoid patients. Among patients of group (1) typhoid fever, there is 12 patients (24%) had serum (TSA), level lies within the range of normal healthy control. 38 patients (76%) their serum TSA levels lies within the range of pathological control while patient of paratyphi group, there is (15) patients (30%) had serum TSA level lies within the range of normal healthy controls had (35) patients (70%) their serum TSA lies within the range of pathological control therefore there is significant overlap in serum (TSA). Level of typhoid patient with that of pathological controls.

The predictive value of serum TSA level in typhoid patients as compared to pathological controls were done by using cut-off value 80 mg/dl calculation were carried out by taking the mean ± 2 SD of normal healthy controls).

Sensitivity, specificity, predictive positively, predictive negativity and efficiency of the test of serum TSA were summarized in table (2).

This table showed that the serum TSA has low predictive value in spite of high sensitivity it has 30% specificity and 52% efficiency in typhi group and para typhi group respectively.

From this result, the TSA in sera of Typhoid patient has severe limitation due to severe overlap with pathological control leading to high false positive results (76%) in typhi group and (70%) in para typhi group and consequently it has low specificity and sensitivity.

These findings consistent with finding reported by (Thougard & Ilmen 1998) that total serum sialic acid is a general disease marker rather than a specific marker and the non specificity of increase makes TSA determination unsuitable as a specific marker and also our result goes with finding reported by (Bircan et al., 1998) that although high TSA in serum but it does not have any clinical significance nor is it important as a diagnostic marker. These findings could be related to that serum TSA concentration does not appear to be associated with the severity of the disease, thus the previously described association between serum (TSA) and infectious disease may reflect the role of mechanism other than the severity of disease (Salomone; 1998).

Although, the TSA normalization is an indicator of probable disease arrest, however is an indication that serial measurement may be more useful than conclusion drawn from isolated value to reflect or correlate with a clinical course (Ujjaini-Khandaria, 1983).

Conclusion

In general it could be concluded that serum (TSA), has limited usefulness as an aid in the diagnosis of enteric fever, however, it may have clinical utility if used in conjunction with other test.

قياس حامض السياليك في المرضى المصابين بالحمى المعوية

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الخلاصة

تم دراسة تركيز حامض الساليك Sialic acid كواسم مصلي للنشاط المرضي في حالات الاصابة بجمي التايفوئيد Typhi Fever. حيث تم قياس تركيز هذا الحامض لدى ٥٠ مصابا بجمي التايفوئيد، ٥٠ مصابا بجمي الباراتايفوئيد Paratyphi Fever، ٥٠ مصابا يعانون من حمى لاسباب مختلفة كمجموعة مرضية ضابطة Pathological Control و ٥٠ شخصا سليما من اية اعراض حمى اتخذوا كمجموعة صحية ضابطة Healthy Control. تبين من خلال النتائج التي تم الحصول عليها ان تركيز حامض السياليك يزداد بصورة ملحوظة لدى الاشخاص المصابين بجمي التايفوئيد والاشخاص المرضى (88mg/dl) و (90 mg/dl) على التوالي، مقارنة بالاشخاص الاصحاء (66 mg/dl). كما اشارت النتائج الى عدم وجود فرق معنوي في تركيز حامض السياليك لدى المرضى المصابين بجمي التايفوئيد والمرضى المصابين بجمي الباراتايفوئيد $P < 0.05$ مما يجعل هذا الاختبار ذا حساسية ضعيفة في التشخيص ولا يمكن اعتباره كواسم كيميوي biochemical marker لدى المصابين بجمي التايفوئيد وانما يمكن اعتباره مؤشر عام لوجود اصابة بجمي.