Serum Total Sialic Acid and Enzymes Levels in Patients with Asthma, Abdominal Bacterial Infections and Bone Disorders

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ABSTRACT

This study was designed to evaluate the clinical application of serum total sialic acid (TSA), total serum protein (TSP) and enzyme activity of alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in serum of 70 subjects. The study involved four groups, the first group (G1) consisted of 17 normal healthy individuals , group 2 (G 2) consisted of 20 patients with asthma, group 3 of 15 patients with abdominal bacterial infection , group 4 involved 18 patients with bone disorders. They were obtained from clinical routine work of different hospitals of Baghdad, Al-Wasity hospital and laboratory center for allergy of asthma. The levels of TSA, TSP and the activity of ALP and GGT were significantly increased in sera of patients with asthma and abdominal bacterial infection ($P< 0.001$) as compared with healthy control group. There was a significant changes observed in TSA, TSP, ALP and GGT in patients with bone diseases. A significant positive correlation between TSA and ALP was observed in patients with asthma and a negative correlation with some cases of bone diseases. A positive correlation between TSA and GGT in patients with asthma and abdominal infection and some cases of bone disease. In conclusion serum sialic acid levels might prove to be sensitive but not necessary specific marker of diagnosis of asthma, abdominal infection and some cases of bone disease.

Keywords: Diseases, Enzymes, TSA, TSP.

مستوى حامض السياليك الكلي والانزيمات في مصل مرضى الربو والالتهابات البكتيرية المعوية وامراض العظام

الخلاصة

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دلت الدراسة على تطبيقات سريرية لقياس حامض السياليك الكلي والبروتين الكلي ومستوى فعالية انزيمات الفوسفتيز القاعدي والكاماكلوتاميل ترانسفريز في امصال سبعون عينة . الدراسة شملت اربعة مجاميع ، المجموعة الاولى تضمنت ١٧ من الاشخاص الاصحاء والمجموعة الثانية تضمنت ٢٠ مريضا يعانون من الربو والمجموعة الثالثة شملت ١٥ مريضا يعانون من الالتهابات البكتيرية في منطقة البطن بينما تضمنت المجموعة الرابعة ١٨ مريضا مصابون بامراض العظام . لقد تم اخذ العينات من مستشفيات مختلفة في بغداد وهي مستشفى الواسطي و مختبر الصحة المركزي ومختبر الحساسية والربو . اظهرت النتائج انه هناك اختلاف احصائي ملحوظ لكل من حامض السياليك الكلي والبروتين الكلي والكاماكلوتاميل ترانسفريز $P<0.001$ عند مرضى الربو والتهابات البكتيرية في منطقة البطن بمقارنتها مع الاشخاص الاصحاء وكذلك هناك اختلاف احصائي ملحوظ لكل من حامض السياليك الكلي والبروتين الكلي ومستوى فعالية انزيمات الفوسفتيز القاعدي والكاماكلوتاميل ترانسفريز لبعض حالات امراض العظام. لقد لوحظ وجود علاقة موجبة بين حامض السياليك الكلي و انزيم الفوسفتيز القاعدي في المرضى المصابين بالربو والالتهابات البكتيرية ولا يوجد علاقة في الحالات المرتبطة بامراض العظام ووجد ايضا ان هناك علاقة بين حامض السياليك الكلي والكاماكلوتاميل ترانسفريز في حالات الربو وامراض الالتهابات البكتيرية في منطقة البطن وبعض حالات امراض العظام . تستنتج الدراسة الحالية ان حامض السياليك الكلي هي طريقة حساسة لوجود حالة مرضية ولكن ليست متخصصة نوعيا لتشخيص حالة مرضية محددة .

INTRODUCTION

Sialic acid (SA), a class of important ketosis that contain nine carbon atoms, it is an acetylated derivative of neuraminic acid (2-keto-5-amino-3,5-dideoxy-D-nonulosonic acid [1]. They are acidic monosaccharide and widely distributed in mammal's tissue, some microorganism and body fluids. N- Acetyl neuraminic acid is the most common form of SA (Figure 1).

Sialic acid significantly bound to glycoproteins, glycolipids, oligosaccharides and polysaccharides, but small amount are free in the body. The unique structure features of this molecule, which includes a negative charge owing to carboxyl group, enable it to play an important role in cellular functions, such as cell – to – cell recognition, adhesion process in an inflammation and transformation to malignancy [2].

SAs are found in cellular secretion and on the outer surface of cells, mostly as terminal components of glycoproteins glycolipids (gangliosides) [3]. The amount of SA on a cell surface is regulated by neuramindase, silalidase and sialyltransferase [4]. SA is exposed to the cellular environment functioning in intrinsic and extrinsic communication, and in defense. The same holds for mucin secretion in which SA not only viscosity but also help to protect epithelia from harmful substances and pathogens [5, 6].

The biological function of SA include: (a) stabilizing the conformation of glycoproteins and cellular membranes; (b) assisting in cell to cell recognition and interaction; (c) contributing to membrane transport; (d) affecting the function of membrane receptors by providing binding sites for ligands; (e) influencing the function, stability, and survival of blood glycoproteins; and (f) regulating the permeability of the basement membrane of glomeruli [7].

Serum SA has been reported as a marker of acute phase response, increased serum SA concentration have been observed in several diseased, e.g., tumors, myocardial infraction, diabetes, inflammatory disorders, and alcoholism [8,9,10]. The clinical usefulness of serum SA determination inherited SA storage diseases is well established. Serum SA is also increased during inflammatory processes because of increased concentrations of richly sialyated acute phase glycoproteins [11]. There are a data suggesting a positive relationship between serum SA and stroke and cardiovascular mortality. Thus several different mechanisms may lead to increased SA concentration in various pathological conditions. The non-specificity of serum SA limits its clinical usefulness. Nevertheless, when combined with other markers, SA concentrations are helpful in disease screening and follow-up, as well as in monitoring of treatment[12].

Asthma is the most common chronic disease in developing countries. Allergy is known to play a significant role in patient with asthma. The prevalence of allergic diseases including asthma has increased significantly over the past 40 years [13]. The reasons for this increase are not well understood but are under active investigation. Asthma is probably not a single disease, but rather a complex of multiple, separate syndromes that overlap. Several classification schemes have been proposed, but many are poorly characterized, with little known about the underlying pathophysiology [14].

Acute abdominal pain generally defined as pain of less than one week's duration and is a common presenting complaint among older patients [15]. The presentation of an older patient with abdominal pain may be very different from that seen in a younger patient [16, 17]. Morbidity and mortality among older patients with abdominal pain are high; evaluation and management often requires admission to the hospital and surgical consultation [18].

Bone is a living, growing tissue. It is made mostly of collagen, a protein that provides a soft framework, and calcium phosphate, a mineral that adds strength and hardens the framework. Throughout one's lifetime, old bone is removed (resorption) and new bone is added to the skeleton (formation). After the age of 30, the bone resorption slowly begins to exceed bone formation [19].

Osteoprosis develop when bone resorption occurs to quickly or when replacement occurs too slowly. Osteoprosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue leading to bone fragility and an increased risk of fractures of the hip, spine and wrist. Men as well as women are affected by osteoprosis, a disease that can be prevented and treated [20]. Osteomalacia is softening of the bone due to lack of vitamin D or a problem with the body and ability to break down and use this vitamin. The softer bones seen in persons with osteomalacia have normal amount of collagen, which gives the bones its structure, but lack the proper amount of calcium [21]. In children this condition is called rickets and usually caused by low level of vitamin D.

The aim of the present study was conducted to evaluate possible correlations of SA with markers of inflammation such as total serum protein (TSP), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) in patients with asthma, abdominal bacterial infections and bone disorders.

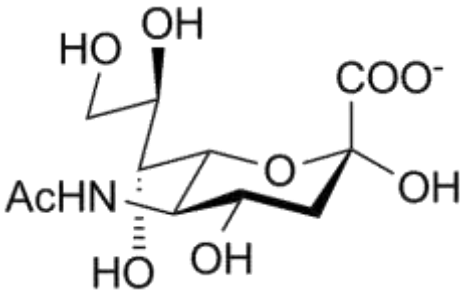

Figure (1): Sialic acid (N-acetyl neuroaminic acid).

MATERIALS AND METHODS

Subjects

The present study enrolled 70 subjects consisted of four groups who were obtained from routine clinical work and investigated by physicians in different hospitals of Baghdad, Iraq from January 2008 to 2009 and divided as follow.

Group 1 consisted of 17 healthy adult blood donors as control subjects.

Group2 consisted of 20 patients with asthma were referred to consultative center for allergy and asthma.

Group 3 consisted of 15 patients with abdominal bacterial infections were attending from healthy center laboratory.

Group 4 consisted of 18 patients with bone disorders were attending from al-wasity hospital for bone diseases, which included three subgroups: the first subgroup consisted of 6 patients with osteoprosis, the second subgroup and third subgroup subgroups consisted of 5 and 7 patients with osteogenic cancer and rickets, respectively. In each case a detailed clinical examination and radiological examination were done and the diagnosis confirmed. Table 1 shows details of the case investigation.

Table (1): Details of cases investigated.

Cases		Number of cases			Age range (years)	Average age* (years)
		Male	Female	Total		
Control		8	9	17	10-50	27.11±10.06
Asthma		10	10	20	19-43	30.95±6.58
Abdominal bacterial infections		8	7	15	4-12	8.06±2.34
Bone disorders	Osteoporosis	1	5	6	36-65	50.66±12.98
	Osteogenic cancer	3	2	5	32-55	40.40±8.84

	Rickets	5	2	7	3-10	7.00±2.94

* Values are Mean ± SD

BLOOD SAMPLES

Five ml of blood from each of the mentioned subjects was controlled from median cubital vein by vein puncture avoiding hemolysis into an evacuated vacuum tube containing clot activator, blood samples were left undisturbed for 30 minutes following which serum was separated from each sample by centrifugation for 3 minutes. The samples were aliquot and kept at – 20 C until analysis was done. All the analysis was carried on serum samples for the measurement of TSA, TSP, ALP and GGT.

MEASUREMENT OF TOTAL SERUM SIALIC ACID

Measurement of total serum sialic acid for control, patients and calibration samples was performed by the resorcinol method by Svenerholm 1958 [22]. Twenty ml for each concentration (10,20,30,40) mg ml^{-1} of calibration samples or serum samples was put in clean and sterile test tube, 980 ml distilled water was added for each tube, the solution mixed and put in ice bath. One ml of resorcinol reagent (2% w/v; coloring SA) was added to each tube. The tubes were put in water bath at 100 ^{0}C for 15 min and then transfer 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. The bottom aqueous layer is removed with a syringe-pipette and the reading is made immediately at 580.

MEASUREMENT OF TOTAL SERUM PROTEIN

Total serum protein was measured by colorimetric method of biuret reaction according to the manufacturer's instruction of Croma test (cat No. 1153005) from linear chemicals, S.L., Spain. The sample was added to an alkaline solution of copper II sulfate. The copper ions from coordinate bonds with the carbonyl and amine groups of the protein. This cause the reagent to change from a sky blue to a purple color. The absorbance of the solution was measured at 540 mn [23].

MEASUREMENT OF ALKALINE PHOSPHATASE

The activity of alkaline phosphatase (ALP, EC 1.1.1.27) was determined using BioMerieux commercial kit (Cat No. 61511) from BioMerieux Sa, 69280 Marcy-I'Etiol, France. Phenol is released by enzymatic hydrolysis from phenylphosphal. The librated phenol was measured by spectrophotometer in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction [24].

MEASUREMENT OF GAMMA GLUTAMYL TRANSFERASE

The activity of gamma glutamyl transferase (GGT, EC 2.3.2.2) was determined by kinetic method using a special kit (BIOLABO SA Reagents, 021602, maziy France). The assay method is based on the transport of gamma glytamyl group from gamma glutamyl P-nitroanilide to glycylglycine by GGT enzyme leaving the yellow product of P-nitroaniline, the analytic method was performed at 30 C by measuring the absorbance change with spectrophotometer [25]. The rate of

formation of P-nitroaniline is directly proportional to GGT activity in the specimens was measured at 405nm.

STATISTICAL DATA ANALYSIS

Data were statistically analyzed using SPSS statistical software (version 11.5) by one way ANOVA test. The values are giving as mean ± standard deviation.

RESULTS

Figure (1) illustrates the value of TSA levels in asthmatic patients, the results revealed a high significant increase ($P < 0.001$), when compared with control group. The data of TSP showed highly significant increase $P < 0.05$, as compared with control group. While, ALP and GGT showed a highly significant increase in their activities ($P<0.001$) when compared with control group.

Figure (2) demonstratesthe levels of TSA, TSP, ALP and GGT activities in patients with abdominal bacterial infection. TSA levels in sera of patients showed a highly significant increase $P< 0.001$ and this elevation was combined with increasing of ALP and GGT, as compared with control group, no significant changes were observed in TSP as compared with control group.

Figure (3 A, B and C) demonstrates the levels of TSA, TSP, ALP and GGT activities in patients with bone disorders. As shown in figure 3 (A) a significant decreased in TSA values $P < 0.05$ and a significant increased with ALP values $P < 0.001$ in patients with osteoporosis as compared with control group. No significant changes were observed in both TSP and GGT as compared with control group.

Figure (3 B) reveales a significant increased in TSA values and ALP values $P < 0.001$ in patients with rickets as compared with control values. No significant changes were observed in both TSP and GGT activity as compared with control values. A similar data was observed in patients with osteogenic cancer (Figure 3 C). There was a remarkable significant rising in the value of TSA and ALP activity $P < 0.001$. No significant differences in TSP and GGT activity as compared with control values.

Figure (4) demonstrates the distribution of serum TSA levels in control and patients with asthma, abdominal bacterial infection and bone diseases. Results in table 1 illustrated the correlation between TSA and the enzyme ALP activity, it was observed significantly remarkable positive correlation in patients with asthma, abdominal bacterial infection and rickets. $P< 0.0001$, $P< 0.006$, $P< 0.0001$, respectively. In patients with osteoprosis, osteogenic cancer there was negative correlation (NS).

Table (2) representes the correlation between TSA and GGT activity, there was highly positive correlation in patients with asthma, abdominal bacterial infection rickets and osteoprosis ($P< 0.0001$, $P< 0.01$, $P<0.001$ and $P< 0.01$) respectively. Patients with osteogenic cancer show a negative correlation (NS).

DISCUSSION

The wide occurrence of sialic acid in various chemical forms linked as monomers or polymers in an outstanding position in a multitude of complex carbohydrate of animal and microorganisms renders them as most versatile function modulators in cell biology and pathology [3]. Increase concentration of

sialic acid in various tissue and fluids has been observed which may be due to at least in part of defective de novo synthesis, transport, storage, catabolism, excretion and or metabolic regulation of sialic acid in cell [26] or may be increased through changes in the biosynthesis and post translation glycosyllation processing of acute protein phase glycoprotein phase in the liver [26].

The results in figure 1, 2, figure 3B and 3C of present study showed there is a significant rising in the levels of total sialic acid in patients with asthma, abdominal bacterial infection, (bone disorder) rickets and osteogenic cancers, the values were 4.43, 2.30, 1.58 and 7.43 folds, respectively of normal limit as compared to healthy individual. Patients with osteoporosis show significant decrease in circulating level of sialic acid; while, in osteoporosis the value was 1.69 lower than normal limit as compared to healthy individual (as described in figure 3A). Ninety percent of sialic acid in normal sera is bound to α and β globulins, and these fractions of serum protein are known to increase inflammatory conditions [27]. Serum sialic acid is also increased during inflammatory process as a consequence of elevated concentration of richly sialyted acute phase glycoproteins [11]. Also, serum sialic acid was reported to be useful parameter of inflammation [28]. Therefore, may be reflecting the reasons of enhanced serum sialic acid concentrations in some pathological conditions.

It is not easy to describe a general role of SA because these monosaccharide participate directly or indirectly in multiple and divers cellular events [5, 6]. This may be possible by their electronegative nature together with their bulky, hydrophilic chemical structure. Sialic operate in the opposite way by being biological recognition sites that is ligands for a great variety of molecules such as hormones, lectins, antibodies and inorganic cations. This protein have been recognized recently as being involved in most important phenomena of cellular and molecular interaction in both physiological and pathological processes [5, 6, 29].

Bronchial asthma is essentially a chronic respiratory disease that manifests itself intermittently as attacks of dyspnea (shortness of breath) and wheezing caused by bronchial spasms. While the basic mechanism of the disease is still poorly understood, all asthma attacks seem to involve a predisposing airway hyperreactivity and the release of a battery of inflammatory mediators that cause bronchoconstriction and mucus hyper secretion [30]. The present results of TSA levels for patients with asthma are in agreement with that reported by Sulaiman et al. [31].

Altered microbial ecology in the gut may produce disease and dysfunction because of the intense metabolic activity and the antigenic nature of bacterial flora. Bacterial enzymes can degrade pancreatic enzyme, damage the intestinal absorptive surface, release the toxins that had previously been bound by conjugation and alter the intestinal milieu in numerous ways, carbohydrate intolerance may be the only symptoms of bacterial overgrowth, and this dietary sugar can be fermented to produce endogenous ethanol. Chronic exposure of the small bowel to ethanol may itself impair intestinal permeability [32]. Bacterial overgrowth is promoted by hypochlorhydria by stasis due to abnormal bowel motility, physical / surgical abnormalities, by immune deficiency or by malnutrition. Any carbohydrate ingested is fermented by the bacteria and results in production of toxic waste products. In the present study, TSA, ALP and GGT

increased in patients with abdominal bacterial, there is very little information about the abdominal bacterial[32, 33].

Bone, being one of the most highly mineralized and complex tissues in the body, is poorly understood in several aspects. The spectrum of non-collagenous proteins within the mineralized bone indicates that these molecules are necessary for matrix structure and mineralization [34]. These matrix components are known to be important for the maintenance of bone structure and calcification. It is thus to be expected that bone disorders would bring about a change in their contents [35]. The results illustrated a highly significant increase in total serum sailic acid levels in patients with rickets and osteogenic cancer as compared to healthy groups as described in Figures (3B), and (3C), and it showed a remarkably significant decrease in patients with osteoporosis, figure 3A. It was demonstrated that the increased levels of carbohydrates of the carbohydrate-protein complexes in sera of cancer patients. As sialic acid occupies terminal position, any change in glycoprotein will account for the changes in sialic acid and vice versa. The elevation of sialic acid contents in cancer patients, could be due to an overall higher amount of sialic acid or due to selective increase in existing specific sialylated sequence or a tumor associated de novo synthesis of specific sialylated sequence [36].

The reduction in sialic acid concentration observed in osteoporosis may be due to either a decrease in the biosynthesis or the protein synthesis may itself disturbed, resulting in low circulating levels of protein bound carbohydrates [37]. The results demonstrated that serum ALP were significantly raised in patient with asthma, abdominal bacterial infection and bone disorder, osteoporosis, rickets and osteogenic cancer, there activities were 1.88, 4.80, 7.95, 6.95, 17.77 above the normal limit as compared with normal healthy individuals. The elevation of ALP activity may be attributed to tissues damage and releasing of enzyme into the blood circulation and also attributed to congestive heart failure, bacterial infection, since ALP enzyme accelerate and initiate chemical reaction essential for life and one of the hydrophilic enzyme which is responsible for breakdown of phosphate ester [38].

The present study demonstrate the considerable increase of ALP activity in patient suffer from bone disorder, osteoporpsis, rickets, this could be due to presence of greater number of blood vessel that form the diseased lead to malformation of bone tissue. Osteoporosis may occur as a complication of endocrine gastrointestinal disease, inflammatory disease and certain drug treatment, poor calcium intake contribute significantly to osteoporosis [20].

Bone ALP is a glycoprotein localized in the plasma membrane of osteoplast. The precise role is unclear although it is essential for mineralization. Bone ALP comprises approximately 50% of total circulating ALP in normal subject [39]. Various studies have reported rise in sialic acid levels and serum ALP activity in bone cancers, malignancy, and osteosarcoma [40], this was in agreement with our results. ALP activity increased in bone cancer due to releasing of enzyme from bone tissue into systemic circulation as a result of tumor necrosis or change of membrane permeability of cancer cells. GGT in bone disorder as compared with normal group not well documented. In present study, a marked increase of GGT in patients with asthma and abdominal bacterial infection, this is due to functional

role of the enzyme which is involved in the transport of amino acid and peptide into cells and glutathione metabolism [41].

GGT is a membrane bound enzyme that occurs in many parenchymatous organs. But appreciable activity of it is only found in kidney, pancreas and small intestine.. As concerns the possible association between GGT and inflammatory process, it should also be considered that GGT is the only enzyme responsible for glutathione catabolism by hydrolysis of gamma-glutamyl bond between glutamate and cystein[42]. The physiological function of GGT enzyme as a source of peptide precursors for intracellular [42, 43]. GGT enzyme that metabolizes extracellular glutathionic and can be induced by various xenobiotics, drugs and all compounds that induce cytochrome.

Results in table 1 showed that there is statistical positive correlation between TSA and ALP in patients with asthma and abdominal bacterial infection (bone disorder) ricket, while there is negative correlation in osteoprosis and osteogenic cancer. Table (2) demonstrate a positive correlation between TSA and GGT in patients with asthma, abdominal bacterial infection, rickets and osteoprosis while a negative correlation in osteogenic cancer. The relationship between TSA and ALP and TSA against GGT need further studies on each pathological case and with a large number of patients.

CONCLUSIONS

Serum sialic acid concentration might prove to be a sensitive but not necessary a specific marker of diagnosis of asthma, abdominal bacterial infection and rickets, bone cancer, since raised level of serum SA have not been reported in several disease. Thus the association between serum SA and infectious disease may reflect the role of mechanism other than the type of disease.

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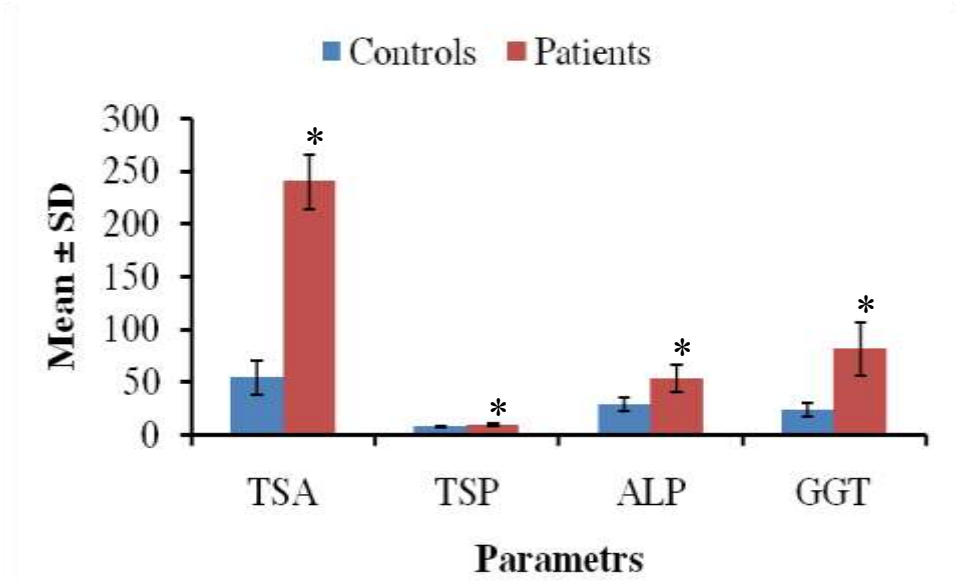


Figure (1): Total serum sialic acid (TSA), total serum protein (TSP), ALP and GGT levels in controls and patients with asthma.* significantly different from control P≤ 0.05.

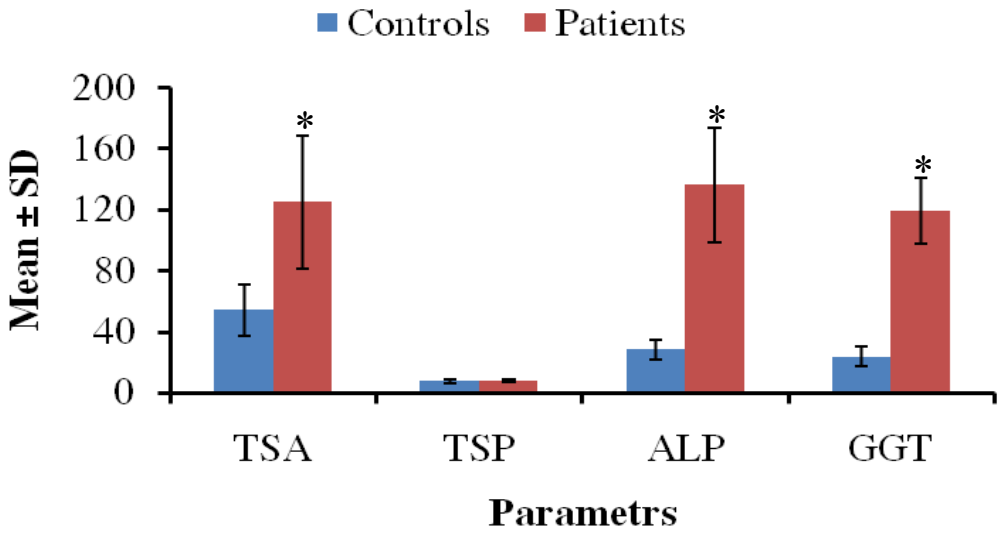
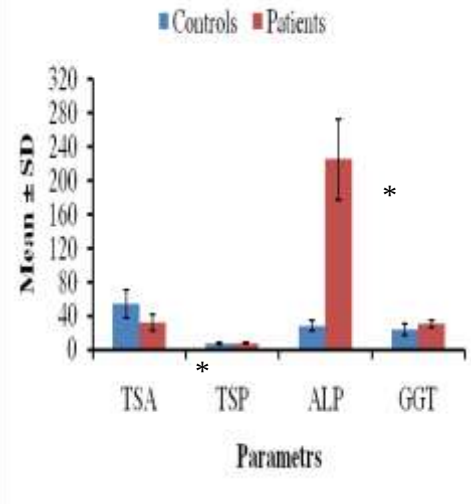
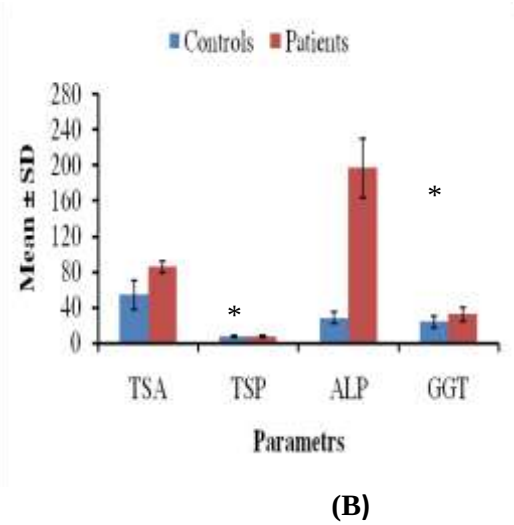


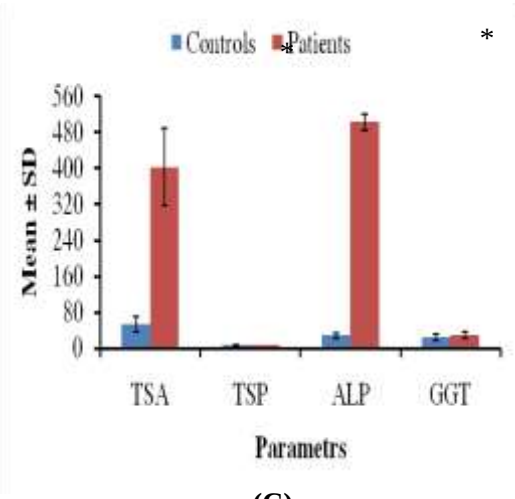
Figure (2): Total serum sialic acid (TSA), total serum protein (TSP), ALP and GGT levels in controls and patients with abdominal bacterial infections.* significantly different from control P≤ 0.05.



(A)



(B)



(C)

Figure (3): Mean total serum sialic acid (TSA; mg/dl), total serum protein (TSP; g/dl), ALP and GGT levels (U/L) in controls and patients with bone disorders. (A) Osteoporosis (B) Rickets (C) Osteoginc cancer.* significantly different from control $P \leq 0.05$.

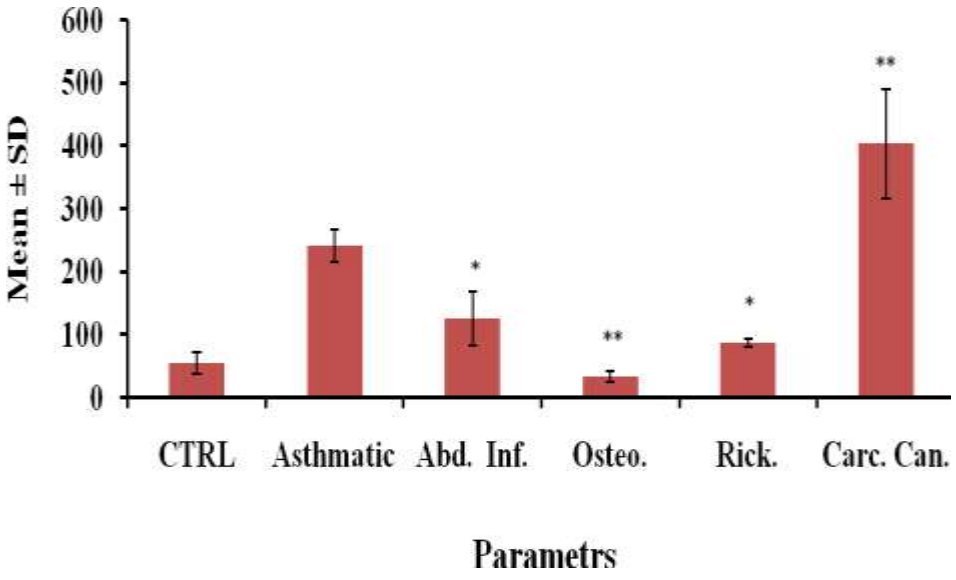


Figure (4): Distribution of serum TSA levels in control and patients with asthma, abdominal bacterial infection, osteoporosis, rickets and Osteoginc cancer. * significantly different from control P≤ 0.05.

Table (1): Correlation of TSA & ALP.

Case TSA&ALP	R	R2	T	Significant -T value
Asthma	0.316	0.1	8.236	0.000
Abdominal bacterial infection	0.136	0.018	3.279	0.006
Osteoporosis	0.266	0.071	2.037	0.111
Rickets	0.7	0.534	9.552	0.000
Osteogenic cancer	0.687	0.472	- 1.254	0.288

Table (2): Correlation of TSA & GGT.

Case TSA& GGT	R	R2	T	Significant -T value
Asthma	0.156	0.024	9.976	0.000
Abdominal bacterial infection	0.279	0.078	2.957	0.011
Osteoporosis	0.772	0.597	3.885	0.018
Ricket	0.25	0.06	7.693	0.001
Osteogenic cancer	0.944	0.891	0.887	0.441