

EVALUATION OF SERUM LIPID ASSOCIATED SIALIC ACID (LASA) CONCENTRATION, MALONALDEHYDE DETERMINATION AND SOME BIOCHEMICAL PARAMETERS IN ARTERIOSCLEROSIS PATIENTS

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Abstract:

The present study was carried out to evaluate the level Lipid associated Sialic Acid (LASA) in patients with cardiovascular Atherosclerosis disease. The study includes three groups the first those who conducted the operation of heart catheter, the second who prepared to enter for catheterizing operation they didn't conducted Catheter operations for them before, while the last group are the normal persons control. The data analysis reflects a significant increase serum LASA level in the patients when compared to normal controls ($P < 0.0001$) for the both patients groups which were done operation catheterize and those are prepared to enter the catheterize. As will as there were shows high significant coloration relation of LASA with some biochemical parameter such as (Bilirubin, HbA1C, thyroxine T4 and CK-MB).

Keyword: Lipid associated sialic acid, Atherosclerosis disease, LFT

Introduction:

Lipid Associated Sialic Acid (LASA), this form represent lipid bound sialic acid, which is present in glycolipids (Robert R. et al. 1993). Glycolipids are widely distributed in every tissue of the body, particularly in nervous tissue such as brain. They occur particularly in the outer login of the plasma membrane, where they contribute to cell surface carbohydrate (Weinstein T. et al. 2000). Lindberg G. et al. (1979), reported that elevated concentration of serum SA is one of investigated risk marker for cardiovascular disease and atherosclerosis.

Biochemical alternation in total sialic acid concentrations has been considerable interest as a potential inflammatory marker for several diseases (Nonda K. et al. 1982, Crooke M. et al. 1993, Okude M. et al 1995). Serum sialic acid levels were associated with an increase risk of acute myocardial infraction (Suer G. et al. 2006, Haq M. et al. 1993), causes ultra-structural changes, initiates inflammatory process and leading to irreversible cell damage which is known as myocardial infarction. Damage to the cell membrane results in the release of elevated level of serum sialic acid has been seen in malignancy, diabetic mellitus, coronary artery disease (Sillanaukee P. et al. 1999).

Acute coronary insufficiency results when the balance between the oxygen requirement and blood supply to the myocardium is disturbed. Cessation of blood flow causes damage to the cell membrane results in the release of intracellular contents and some membrane

components like sialic acid (Merat A. et al. 2003). A recent epidemiological study showed that mortality from cardiovascular diseases was higher in population with high concentrations of sialic acid (Gokmen S. et al. 2000). It has also been demonstrated that dyslipidemia, smoking, hypertension are important modifiable risk factors for cardiovascular disease and associated with high serum sialic acid (Lindberg G. et al. 1991). Various studies indicate increased LDL desialylation is associated with increased peripheral atherosclerotic lesions. This in turn suggests a possible role of sialic acid in atherosclerosis and also its association with dyslipidemia. Hence, comparison of serum sialic acid levels with atherogenic index may be used as predictor of atherosclerosis (Chappey B. et al. 1998). This study was carried out to evaluate the level LASA in patients with atherosclerosis disease.

Materials and Subject

The number of sample which taken in this present study are more than ninety samples, but Patients with diabetes, renal failure, previous history of angina or MI, malignancy, autoimmune disease, acute infection, taking lipid lowering drugs or antioxidant supplements were excluded from the study only fifty patients shows have atherosclerosis disease only, the diagnosis and assay were done by doctors in surgical specialty hospital cardiac center in Hawler city, from May. 2014 to spt. 2014, they are divided into two groups according their history of patients, the first group those who

conducted the operation of heart catheter, included 36 patients (females and males) the age range are between 25 -79 year, and the second group which was those diagnosis and assayed their disease and prepared to enter for the operation catheterizing included 14 patients (females and males). The study also included 28 subjects of normal healthy control (females and males) the age range (25-55 years) with socioeconomically matching was taken having no history of heart disease and it was a case controlled study conducted in the department of chemistry - Science College at Salahaddin University.

Blood Sampling

The blood was allowed to coagulate at room temperature and centrifuged at 3000 rpm for 20 min. The resulting sera were separated. The sera were then stored at -20°C when it was not used immediately. The stability studies showed that the following parameter LASA was constant under these conditions, for up to four months (Gabrlel L. et al.1988).

Biochemical Tests

The biochemical tests includes serum fasting LASA, cholesterol, triglyceride, HDL, LDL, GPT, GOT and ALP and other parameter like blood sugar, HbA1C, urea, uric acid, creatinine and alkaline phosphatase.

Statistical Analysis

Statistical analysis was done by using student t- test. All the data were presented as mean \pm standard deviation. 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 unpaired and two tail) and taking P-value 0.05, or less as the lowest limit of significance.

Instruments

1. Spectrophotometer, LKB, Model 4050
2. Hotplate (Stuart Scientific Co.LTD No.5371 England)
3. Centerifuge centra 4, International (IEC).
4. waterbath (Memert Gm bH+ Co. KG D 91126)
5. Oven
6. Analytical balance

Chemicals

All common laboratory chemicals and reagents used in this study were of analar grade unless otherwise specified, and were obtained from the following companies:

1. NaOH, NaCl, methanol, ethanol, chloroform, perchloric acid, and butyl acetate. In Fluka Company, U.K.,
2. Orcinol, resorcinol, H₂SO₄ Cons., and HCl Cons. In Hopkins and William Company,
3. Phosphotungestic acid in Riedel- Deherfform Company,
4. Sigma Chemical Company, for NANA.

Lipid associated sialic acid determination

The method for LASA determination was essentially as described by Katopedis (Katopedis N. et al.1980). The glycolipids were extracted from serum by addition of chloroform and methanol (2:1). The supernatant were transfer to other test tube then the lipid precipitated by adding phosphotungestic acid, the precipitate was dissolved by water after formation of Chromogen by addition of resorcinol-HCl reagent then the chromogen extracted and read as in TSA assay (Svennerholm L.1975, Nopnda K.et al.1982).

BIOCHEMICAL TEST

Serum lipid profile, GPT, GOT urea, sugar and bilirubin were analyzed by fully automate Random access biochemistry analyzer (Cobas integra 400), using commercially available kits of Randox laboratories. Atherogenic Index was calculated as total cholesterol- HDL cholesterol/ HDL cholesterol. Serum CKMB is measured by semi auto analyzer, using commercially available kits.

Determination of serum malondialdehyde (MDA):

The level of serum MDA was determined spectrophotometrically with a TBA solution. In brief to 150 μl serum sample added the followings: 1ml trichloroacetic acid 17.5 %, 1ml of 0.66 % thiobarbituric acid (TBA), mixed well by vortex, incubate it in boiling water for 15 minutes, and then allowed to cool. Then add 1ml of 70 % TCA, and let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and take out the supernatant for scanning spectrophotometrically (Weinstein T. et al.2000).

Results:

The results of the LASA of the patients with Atherosclerosis Patients (36) in addition to healthy controls (28) are presented in Table (I, 2, 3). Which revealed that there is a significant

increase ($P < 0.0001$) in the LASA level in the serum of patients with Atherosclerosis Patient as compared with healthy control Fig.(1). The following tables show all results in this study:

Table (1) Standard t-test LASA mg/dl level of control and Atherosclerosis Patient who conducted the Catheterized.

	Control		catheterized	
	LASA mg/dl	age	LASA mg/dl	age
No.	28	28	36	36
Range		25-62		20-80
Minimum	3.989	25	26.6	20
Maximum	52.39	62	255.3	80
Mean	24.67	27.8	122	56.28
SD ±	12.92	8.62	78.4	12.56
SE	2.441	1.63	13.07	2.093
T-test	6.4			
Probability	P< 0.0001 High significant			

In the table (2) shows the significant increase in the serum LASA level in the Atherosclerosis patients which are prepared to enter catheterize operation compared to the normal control persons ($P < 0.0001$).

Table (2) Standard t-test LASA mg/dl level of normal control and Atherosclerosis Patients who are prepared to enter into operations Catheterized

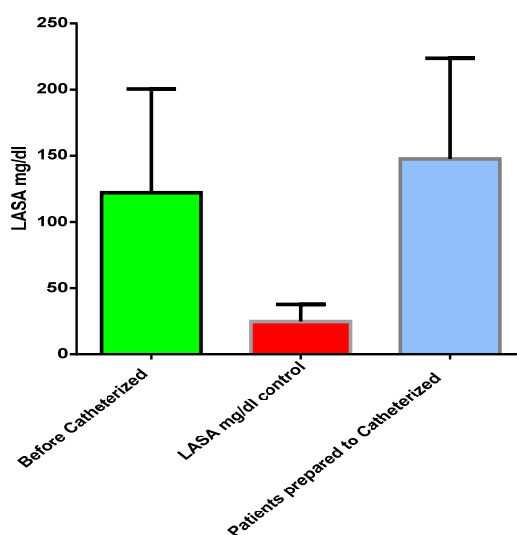
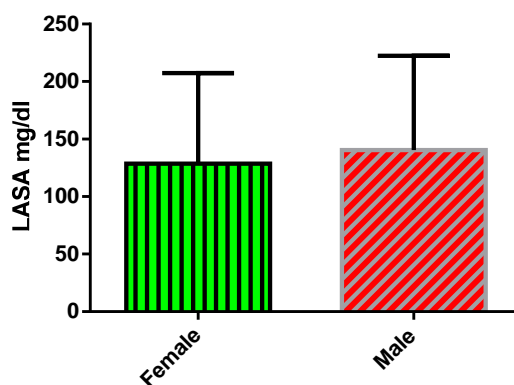
	Control		Prepared to Catheterize	
	LASA mg/dl	Age	LASA mg/dl	age
Number	28	28	14	14
Range		25-62		25-77
Minimum	3.989	25	26.60	25.000
Maximum	52.39	62	239.4	77.00
Mean	24.67	27.75	147.6	57
SD ±	12.92	8.622	76.09	21.10
SE.	2.441	1.629	20.34	5.640
T-test	8.4			
Probability	P< 0.0001 High significant			

Table (3) Correlations of LASA with some biochemical parameter for Atherosclerosis Patients who were operations Catheterized.

Biochemical parameters	Correlations LASA mg/dl	P- value
MDA	-0.1835	0.284
Age	-0.2015	0.239
Bilirubin	-0.6285	0.052
HbA1C	0.7906	0.034
THYROXINE T4	0.9036	0.035
CK-MB	-0.8562	0.064

There are high positive correlations between LASA with cholesterol, HbA1C, THYROXINE T4, Bilirubin and LDL for the patients after catheterize, while negative correlation found with GOT, GPT and HDL as shown in table (3).

There are no difference's between female and male LASA concentration as shown in Figure (2).

**Figure (1):** T-test of (LASA) in the serum of Atherosclerosis patients and the control group.**Figure (2)** T-Test of LASA for Male and Female patients

Discussion

Sialic acid is usually bound to glycoproteins, glycolipids, oligosaccharides, polysaccharides and a small amount is free in the body (shivanada Nayak, Losley R.2006). In this study the reference material consisted of selected sample of Atherosclerosis Patients in Erbil city at (central of heart operation). The primary goal was to determine the reference serum LASA concentrations for women and men if possible. But the number of samples quantity not aloud that, Also studied the effect of age, blood pressure, smoking, and hormonal factors on serum LASA and malondialdehyde. Serum LASA levels were associated with an increased risk of acute myocardial infarction (Suer G. et al.2006, Haq M. et al.1993, GitllM.et al.2004) .This study was carried out to evaluate the level LASA in patients with Atherosclerosis disease patients and shows significant increase in the patients when compared to normal controls ($P < 0.0001$) for two groups of the patients which are done operation catheterize and which are prepared to enter the operation catheterize (table 1, 2). 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 (Okude M. et al 1995), and rheumatoid arthritis (Maritta P. et al.1999). The mean serum LASA concentrations did not differ between female and male subjects as shown in Figure (2) T- Test of LASA Male and Female, and they were in the range of 10 – 239 mg/dL, which is in accordance with earlier finding with the Sillanaukee P. et al.1999. Also the present study found that smoking not effect on increased the serum LASA concentration in both male and female, all though in the present investigation also observed LASA level was significantly high positive correlation with cholesterol , HbA1C, ThyroxineT4, Bilirubin and LDL levels in serum of patients who done catheterize as compared to normal table(3), this marked increased in LASA level may indicate the deformation has been occurred in the cell.(Dutt M. et al. 1975), reported that raised serum TSA levels as the earliest marker of raised

atherogenic index. It has been suggested that desialylation of LDL is an atherogenic modification, taking place in the circulation, since sialic acid poor LDL has been found in blood, this finding agreed with the reported (Anujparkash.et al.2011). While, negative correlation found with GOT, GPT and HDL as shown in table (3).

Conclusion:

The changes in serum lipid associated sialic acid may be used as supporting tool in the identification of Atherosclerosis disease properties. More investigations are required to obtain a complete explanation of the causes of the increase in LASA in the tissue and serum of patients; and the follow up studies are recommended.

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ههلسهنگاندنی پهیتی چهوری بهیه کهوه لکاوی ترشی سیالیک له سیره م و دیاری کردنی پهیتی مالون نه لدهاید وه ههندی پتورهی کیمیای ژبانی له تووشبه کانی نه خووشی رهق بوونی خویشبه ره کان دل

پوخته:

له م تویشنه وه دا دهسکرا به ههلسهنگاندنی ریژهی چهوری بهیه کهوه لکاوی ترشی سیالیک له سیره م له تووشبوانی نه خووشیه کانی رهقبونی خوین به ره کانی دل. نه م تویشنه وه دا سی کومه له له خووه دهگری، به که م کومه له بریتینه له وانیه کهوه تووشبونه به نه خووشی دل و وه نهشته رگه ری قهسته ریان بو کراوه وه کومه له دووم بریتینه له وانیه کهوه ناماده کراون بو چوونه ناو نهشته رگه ری قهسته ره و رهقبوونی خوین به ره کانی دل، هه رچی دوا کومه له به بریتینه له کونترول (کهوه کهسی ساغ و نورمالن). دوا ی شیکردنه وهی داتا کان به دیار کهوت کهوه زیاد بوونه کی بهرچاوه هیه له پهیتی چهوری بهیه کهوه لکاوی ترشی سیالیک (LASA) له سیره می کهس تووشبه کان بهراود به کهسه نورماله کان ($P < 0.0001$)، بو ههردوو کومه له، کومه له به که م بریتینه له وانیه کهوه تووشبونه به نه خووشی رهقبوونی خوین به ره کانی دل و وه نهشته رگه ری قهسته ره بو کراوه وه کومه له دووم که بریتینه له وانیه کهوه ناماده کراون بو چوونه ناو نهشته رگه ری قهسته ره و رهقبوونی خوین به ره کانی دل. وه ههروه ها به دیار کهوت کهوه به ریژهیه کی بهرچاوه په یوهندی هیه له نیوان پهیتی چهوری بهیه کهوه لکاوی ترشی سیالیک و ههندی پتورهی کیمیای ژبانی له تووشبه کانی نه خووشی دل وهک بلرویین شه کره که له که بووه کان و تایروکسین T4 وه کریاتینین کاینیس (Bilirubin, HbA1C, thyroxine T4 and CK-MB)

تقیم ترکیز حمض السیالیک الدهنی، مالونوالدهاید و بعض التحاليل الكيمياء الحياتية في مصل المرضى تصلب الشرايين
الاعوية القلبية

الخلاصة:

الدراسة الحالية جاءت لتقييم مستوى حمض السیالیک الدهنی في مرضی المصابین بتصلب الشرايين الاعوية القلبية. حيث تتضمن الدراسة ثلاثة مجاميع، المجموعة الاولى التي خضعت لعملية القسطرة، المجموعة الثانية الذين تم تهيئتهم لاجراء عملية القسطرة ولم يجرى لهم القسطرة من قبل والمجموعة الاخيرة هم الاشخاص الاصحاء كمجموعة سوي. ان تحليل البيانات تعكس الزيادة الملحوظة في مستويات حمض السالیک الدهنی (LASA) في المرضى لكلا المجموعتين عند مقارنتهم مع المجموعة السوي ($P < 0.0001$)، وبالإضافة وجد ان هناك علاقة ارتباطية بارزة لحمض السیالیک الدهنی (LASA) مع بعض التحاليل الكيمائية الحياتية مثل (بيلروبين، تايروكسين T4، وكرياتينين كائينز - النظير القلبي والسكر التراكمي) اي (Bilirubin, HbA1C, thyroxine T4 and CK-MB).