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Determination of sialic acid and biochemical parameters level in β-thalassemic patients

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الخلاصة:

يختص هذا البحث بدراسة التغيرات الكيمياحياتية التي تحدث في المرضى المصابين بفقر دم البحرالأبيض المتوسط الرئيسي (بيتا ثلاسيميا ميجر), ولقد تم تحديد هذه التغيرات في عشرين طفل (ثلاثة عشر طفل منهم مصاب بفقر دم البحر الأبيض المتوسط الرئيسي وسبعة أطفال أصحاء غير مصابين).

المتغيرات التي تم دراستها في هذا البحث هي :- حامض السياليك الكلي في مصل الدم, معدل ترسيب كريات الدم الحمراء والبروتين المصلي المخاطي الكلي.

ولقد أكتشف من خلال هذه الدراسة أن هناك نقصان في مستوى حامض السياليك الكلي والبروتين المصلي المخاطي الكلي في المرضى المصابين بفقر دم البحر الأبيض المتوسط الرئيسي مقارنة بالأطفال الغير المصابين بينما هنالك زيادة في معدل ترسيب كريات الدم الحمراء في المرضى المصابين بفقر دم البحر الأبيض المتوسط الرئيسي مقارنة بالأطفال الغير المصابين.

Abstract:

This research was concerned with the biochemical changes that might occur in patients with β-thalassemia major, multiple determinations were obtained 20 subjects: - 13 children with β-thalassemia major and 7 normal children (as normal healthy control).

The parameters deduced from this study includes:- serum total sialic acid (TSA), erythrocytes sedimentation rate (ESR) and seromucoid total protein (TP).

From the data obtained for these parameters investigate the decrease in serumTSA and TP of thalassemic patients compared to normal individuals, while the ESR levels increase for patients as compared to other individuals.

Introduction:

Biosynthesis of sialic acid and β -thalassemia:

The term sialic acid comprise a large family of N- and O-substituted neuraminic acid derivatives as components of glycoconjugates (5-amino-3,5-dideoxy-D-giycero-D-galacto-nanulosonic acid) ($C_9H_{17}O_8N$) [1,2] which occur widely in nature, as present in a variety of tissues and body fluids of higher animals and some bacteria, but not in plants or lower invertebrates [3] .

Sialic acid is formed from glycogen by interaction of fructose-6-phosphate with glutamine [4], followed by acetylating by acetyl-CoA in the liver.

Three types of β-thalassemia could be recognized: - β-thalassemia major (Cooley's anemia), intermediate (HbA_1) and β-thalassemia minor [5,6].

On other hand, the diagnostic features of this disease were evidence of severe red cells dysplasia, erythroblastosis and absence or gross reduction of amount of HbA.

Several studies have proposed possible causes lead to destruction of red cells for β-thalassemic patients, these are:-

1. The RBC_S in β-thalassemia are hypochromic, microcytic and targeted (indicating an excess of cellular surface over the reduced volume) [7].
2. They have abnormal and irregular shapes with fragmented forms, buds, and spicules, which strongly suggests that plastic changes have occurred in red cells membrane.
3. Indeed, a number of biochemical alterations have been identified in thalassemia erythrocytes including increased calcium concentration, alterations in membrane phospholipids asymmetry and cholesterol content [8].
4. Studies on phagocytosis of thalassemia erythrocyte suggest that alterations of thalassemia erythrocyte membrane are evident on young erythrocytes rendering them more recognizable as compared to phagocytosis of normal erythrocytes [9,10].

Geographic distribution:

The thalassemias are most common in the areas of the world historically affected with endemic malaria, including the Mediterranean shores, the Arabian Peninsula Turkey, Iran and Southeast Asia. The high frequency of thalassemia in these areas may be attributed to the fact that the heterozygous thalassemias enhance resistance to malaria [11].

Treatment of B-thalassemia [12,13]:

The treatment of β-thalassemia major is given in (Table -1):

Problem	Management
Erythropoitic failure	1.Allogenic bone marrow transplantation. 2. Hypertransfusion. 3. Folic acid (5 mg) daily.
Iron overload	Iron chelating therapy
Splenomegaly causing mechanical problems, excessive transfusion required.	Splenoectomy performed as late as possible.

Table -1: treatment of β-thalassemia major.

Materials and Methods:

Materials and apparatus:

All common laboratory chemicals or reagents were analyzed grade unless otherwise specified and were obtained from the following companies:-

1. Resorcinol, $CuS0_4$, HCl, H_2S0_4, D (+)-Galactose, D (+)-Mannose, Folin-Cosi Culteen, and tris (hydroxyl methyl amino) methane from BDH Company.
2. Nacl, Na_2C0_3, NaH_2P0_4, Bovine serum albumin (BSA) from Fluku Company.
3. Na, K-tartarate, n-butanol, sialic acid from Sigma Company.

Instruments:

a. Spectrophotometer UV (160-030) shimdazu SP3-spectrophotometer.
b. PH-meter, model 720-philips.
c. Analytic sensitive balance, Sartorious.
d. Thermostat water bath, LKB.
e. Cooling centrifuge, Sigma-202 MK.
f. Magnetic stirrer, Hot-Plate-Stuart.

Blood collection:-

Venous blood was sampled from 13 subjects of both sexes, whose β-thalassemia major conditions were confirmed by electrophoresis of the hemoglobin (HbF %). Blood was also collected from 7 normal healthy subjects of both sexes. Both groups were within the same age range (10-30 years).
For studies of TSA and TP from 20 subjects (patients and controls) were used and whole blood of the same groups were used to measure ESR(Table -2)

Group	Subjects No.	Female No.	Male No.	Age range
Thalassemic patients	13	6	7	15-25
Normally healthy control	7	5	2	10-30
Total	20	11	9	

Table- 2: the host information of all patients and healthy subjects studies.

Serum specimens:

The blood was allowed to coagulate at room temperature and centrifuged at 3000 rpm for 20 minutes; the resulting sera were separated and frozen at-20C˚ until assayed.

Biochemical tests (ESR, TP, and TSA):

- The total sialic acid was determined according to the method [14].

1. 20 µL of sera sample or standard NANA solutions and 980 µL of deionized water placed in test tubes vortexed and placed on ice.

2. To each assay tube, 1 ml of resorcinol reagent (2% w/v) was added, and placed in a 100C boiling water bath for exactly 15 minutes followed by 10 minutes on ice-bath.
3. 2ml of butanol, was added to each tube then vortexed and centrifuged for 10 minutes at 3000 rpm, the extracted chromophore was read at 600 nm.

- The ESR-test measures the sedimentation rate of aggregated red cells in plasma by Westergren method [15,16], the Westergren tube is a straight glass tube 30 cm in length and 2.5 mm in diameter, it is calibrated in mm from 0 to 200 mm.
- Erythrocytes ghosts total protein (TP) determination:-

For protein determination, the RBC_S ghosts suspension (125 µL) was dissolved in 0.1 N-NaOH (125 µL), and analyses were performed according to the method published in literature [17].

Results and Discussion:

Serum TSA mg/100ml	Normal healthy control	Thalassemic patients
Sample size n_1	7	13
Mean ± SD	20 ± 5	9 ± 2
Standard error of mean	2.30	1.01
T-test	----------	8.35
P-value	----------	< 0.0007

Table-3: Biostatistical calculation and T-test for TSA levels in sera of normal healthy controls and thalassemic patients.

S.TP(mg/dl)	Normal healthy controls	Thalassemia patients
Sample size	7	13
Mean ± SD	7.5 ± 0.40	5.90 ± 0.30
Standard error of mean	0.055	0.130
T-test	----------	3.15
P.value	----------	< 0.033

Table-4: Biostatistical parameters for TP levels in sera of normal healthy and thalassemic patients.

Biochemical test	Sensitivity	Specificity	Positive(+ve) predictability	Negative (-ve)	Efficiency*
s.TSA	80%	90%	87%	86%	80%
s.TP	75%	62%	71%	79%	81%
ESR	90%	68%	78%	68%	80%

*Efficiency of test= % of patients correctly classified as diseased and non-diseased[18].

Table-5: The predictive values for the overall biochemical markers in thalassemic diseased patients.

TSA (mg /dl)	%HbF
25	95
28	93
30	92
35	90
40	88
45	82
50	76

Table- 6: Variation of TSA (mg/dl) with HbF %for thalassemic patients.

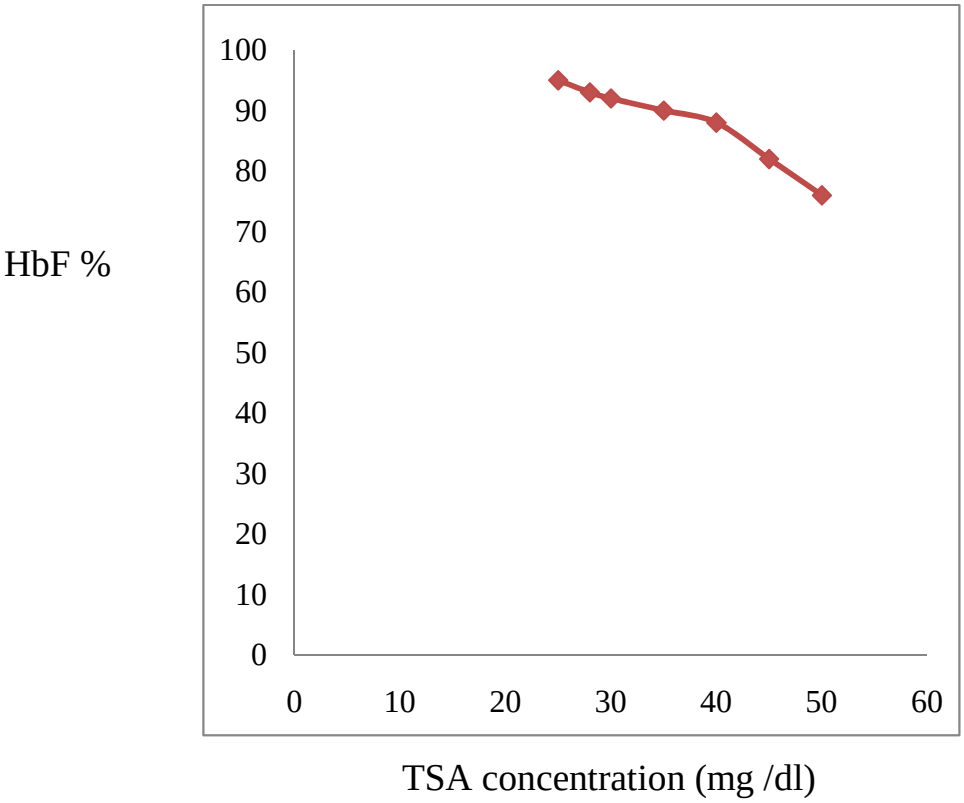

Figure-1: The correlation between serum TSA and HbF% for thalassemic patients.

Erythrocyte Sedimentation Rate (ESR):

From the data obtained of ESR for thalassemic patients, it is important to deduce the mean ± SD of ESR levels expressed as mm/h [19] in sera of normal healthy controls and thalassemic patients.From the biochemical statistical in (Table- 5), the 80% efficiency refers to the significance of this parameter to diagnosis of this disease compared to other biochemical parameter [20].

Serum total sialic acids (TSA):

This study show significant decrease in total sialic acid concentrations in the serum of thalassemic patients (30 mg/dl) when compared to normal healthy controls (50 mg/dl).The statistical analysis (Table -5 and 6) using a paired T-test shows difference between the means of healthy control and the level of thalassemic patients (P< 0.0007) (Table-3); there is a similar decrease in membrane sialic acid in β-thalassemic disease [21,22]. Gabriel and Workers also found a similar decrease in serum total sialic acid in sickle cell anemia [23].

Erythrocyte ghosts sialic acid (TP):

The results presented in this work, revealed highly significant decrease of thalassemic membrane sialic acid concentration (18 µg/mg protein) as compared to normal erythrocyte controls (35 µg/my protein). A paired T.test showed a significant difference between the means control and the mean disease S.TP (P<0.003) (Table- 4). These findings were in contrast with these reported by calatromin etal [24].

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