A STUDY OF SERUM HAPTOGLOBIN LEVELS IN CASE OF PRIMARY IDIOPATHIC HYPOTHYROIDISM

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SUMMARY: Twenty five patients with clinical and laboratory diagnosis of Primary Idiopathic Hypothyroidism were subjected to serum haptoglobin estimation before the commencement of replacement therapy with L-thyroxine. Serum haptoglobin levels were observed to be significantly reduced or absent in these patients ($p<0.05$). The serum levels of haptoglobin in these patients showed a marked rise during the treatment and follow-up period of four and eight weeks ($p<0.05$). The role of serum haptoglobin as the cheapest and sensitive marker for laboratory diagnosis of Primary Idiopathic Hypothyroidism and its role in the management of these patients are discussed.

Key Words: Hypothyroidism, haptoglobin, T_3, T_4, thyroid stimulating hormone.

INTRODUCTION

Haptoglobin is the name given to a family of α_2 glycoproteins that bind hemoglobin. It acts as the principal factor affecting the renal threshold for hemoglobin (9). It is an acute phase protein and its concentration rises by 25% or more in the plasma in the first seven days following tissue damage and in the acute phase of various inflammatory states (8,13). However, anhaptoglobinemia is usual in newborn. Concentrations are measurable by about three months of age and increase gradually to adult levels by age twenty years (4,14). Decreased concentrations are observed in hemolytic anaemia, ineffective erythropoeisis, liver disease, hereditary anhaptoglobinemia, pregnancy and with estrogen therapy. The usefulness of acute phase protein measurements, including haptoglobin, in clinical practice has been recently emphasized by Thompson *et. al.* (16).

The first case of acquired anhaptoglobinemia was reported by Masam Kuriyama *et. al.* in panhypopituitarism which was completely reversed by the administration of hydrocortisone (11). Khazi *et. al.* detected and reported acquired anhaptoglobinemia in a case of insulin dependent diabetes mellitus with primary hypothyroidism (6).

The hospital records and recent studies (1,3) show that the incidence of primary idiopathic hypothyroidism (PIH) is quite high among the population. Therefore, it was thought worthwhile to study the levels of serum

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haptoglobin in PIH and to assess the usefulness of this estimation as a simple, rapid and cheap laboratory indicator for the diagnosis, treatment and follow-up of the patients with PIH keeping in view the cost of thyroid hormone kits and time period needed for their analysis.

MATERIALS AND METHODS

Patients

Twenty five Libyan patients (10 males and 15 females) in the age group of 35-55 years with clinical diagnosis of PIH attending the referral Endocrinology Clinic at Benghazi, Libya, were included in the study. The most common presenting clinical features of patients were generalized weakness, lethargy, dry skin, edema of eyelids, change in voice, constipation and hypersensitivity to cold.

Specimen collection

Following clinical diagnosis of PIH, purpose of the study was explained to each patient. Ten ml of whole blood was collected from the antecubital vein of each patient in fasting state (PIH, I°). One half of the specimen was put in an EDTA vial for routine hematological investigations and the other half in a sterile silicone coated plain tube. An aliquot of separated serum was immediately taken and kept frozen at -70°C until analyzed for thyroid hormones. Rest of the serum was used for haptoglobin estimation and also for liver function tests, estimation of iron and iron binding capacity, Coomb's test, immunoglobulin estimation and protein electrophoresis. Patients with any clinical or laboratory evidence of intravascular hemolysis, liver dysfunction, infection or inflammation were excluded from the study. Blood specimens were again collected from each patient at four weeks (PIH-II°) and eight weeks (PIH-III°) following replacement therapy with L-thyroxine.

Control subjects (CS)

Twenty five age and sex matched healthy Libyans blood donors and volunteers were taken as a control group for the estimation of serum thyroid hormones and haptoglobin levels.

Estimation of Serum Thyroid Hormones (TFT)

Serum levels of total T_3 and T_4 were estimated by using Radioimmunoassay kits "^{125}I-T_3-COATRIA' and "^{125}I-T_4-COATRIA" respectively supplied by bioMerieux Company, France. Serum TSH level was measured by Amerlex-RIA-kits of Amersham, UK. These commercially available kits are based on the principle of competitive inhibition. Controls at three levels were used with each assay. The intra- and inter-assay coefficients of variation were less than 10%.

Table 1 : Serum thyroid function tests and haptoglobin levels in patients with primary idiopathic hypothyroidsm and in control subjects.

Subjects (N=25)	THYROID FUNCTION TEST			Haptoglobin level (mg/dl)
	T3 (nmol/L)	T4 (nmol/L)	TSH (min/ml)	
At diagnosis (PIH-I°)	0.40 ± 0.22	10.20 ± 4.83	42.56 ± 9.79	23.72 ± 13.23
At 4 weeks of therapy (PIH-II°)	1.42 ± 0.47	63.20 ± 20.31	13.26 ± 7.73	58.92 ± 14.85
At 8 weeks of therapy (PIH-III°)	1.70 ± 0.32	95.04 ± 17.78	5.64 ± 3.47	118.48 ± 45.97
CONTROL (CS)	1.76 ± 0.41	121.48 ± 30.21	3.43 ± 0.98	153.72 ± 68.23

Values expressed as Conc., Mean ± SD

Estimation of Serum Haptoglobin

Serum haptoglobin was estimated by Radial immunodiffussion (RID) method as described by Mancini *et. al.* using RID plates obtained from bioMerieux, France (10). The control serum 'Immunotrol' supplied by bioMerieux was included with each assay. The intra- and inter-assay coefficient of variance were < 3 %. The detection limit of the procedure was ≥ 10 mg/dl.

Table 2: One way analysis of variance of data shown in table 1.

Parameters	SUBJECTS	(CS, PIH-I°, PIH-II°, PIH-III°)	
	F-Test	d.f	P
T3	71.25	3.96	<0.001
T	15.56	3.96	<0.001
TSH	193.4	3.96	<0.001
Hapt.	50.23	3.96	<0.001

P>0.05 = Not Significant

Table 3: Student's t-test applied to serum haptoglobin and thyroid hormone levels at different periods of replacement therapy with L-thyroxine.

SUBJECTS	PARAMETERS							
	T_3		T_4		TSH		Hapt.	
	t	p	t	p	t	p	t	p
1. CS vs PIH-I°*	14.78	<0.001	18.24	<0.001	-5.62	<0.001	9.38	<0.001
2. CS vs PIH-II°*	1.39	>0.1	8.0	<0.001	-6.32	<0.001	6.8	<0.001
3. CS vs PIH-III°*	0.582	>0.5	3.78	<0.001	-3.0	<0.005	2.1	<0.05
4. PIH-I° vs PIH-II°**	0.558	>0.5	12.73	<0.001	11.78	<0.001	8.8	<0.001
5. PIH-II° vs PIH-III°**	6.2	<0.001	5.9	<0.001	4.5	<0.001	6.1	<0.001
6. PIH-I° vs PIH-III°**	1.04	>0.01	23.0	<0.001	17.8	<0.001	9.9	<0.001

(CS: Control subjects; PIH-I : Pt at diagnosis; PIH-II : After 4 weeks of therapy; After 8 weeks of therapy).

* Student's t-test

** Paired t-test

P>0.05, NS; Not significant; d.f = 25+25-2 = 48

Statistical analyses

The statistical significance of the results were evaluated by using student's t-test. Paired t- test and one way analysis of variance (7).

RESULTS

The results of the thyroid function tests (TFT) and serum haptoglobin levels and their statistical analysis are stated in Tables 1, 2 and 3. All twenty five patients showed hypohaptoglobinemia. The one way analysis of variance indicated significant differences in serum TFTs and haptoglobin levels in PIH-I°, PIH-II° and PIH-III° (Table 2). As TFTs improved with L-thyroxine therapy, serum haptoglobin levels were also significantly raised at 4 weeks (PIH-II°) and at 8 weeks (PIH-III°) (Table 3).

DISCUSSION

All patients with clinical features of PIH confirmed by the presence of low serum T_3 and T_4 levels and elevated serum TSH levels, were found to have hypohaptoglobinemia. There was a significant rise in serum haptoglobin levels following replacement therapy with L-thyroxine. It has been shown in some animal experiments that the addition of glucocorticoids significantly increased the rate of haptoglobin synthesis in the acute phase of inflammation which might be due to induced synthesis of hepatic RNA for plasma acute phase proteins' (5,15). It seems that L-thyroxine, like glucocorticoids, could also affect haptoglobin synthesis. Miller and Griffin (12) showed that addition of 4 micrograms of thyroxine to perfusion fluid increased haptoglobin synthesis in normal liver and this increase was higher than that seen in perfusion with insulin and cortisol. Livers of hyperthyroid donors were found to synthesize more haptoglobin than those from normal or hypothyroid donors (12).

There is a general decrease in protein biosynthesis in PIH. Hormone replacement stimulates protein biosynthesis. Thyroid hormone binds directly to the nucleus of the receptor cells and affects transcriptional

and posttranscriptional events leading to rise in various proteins including haptoglobin.

L-thyroxine probably also induces expression of cytokine receptors (IL-1, IL-6 and TNF) in hepatocytes which in turn may stimulate posttranscriptional and translational events leading to production of haptoglobin (2). This hypothesis is based on the fact that cytokines, thyroid hormones, and neurotransmitters all appear to be integrated in a highly potent immunoregulatory circuit.

As PIH is a common clinical entity, a simple, cheap, rapid and a sensitive marker is needed for its laboratory diagnosis. Haptoglobin fulfilles all these criteria and should be introduced as a laboratory marker for diagnosis, treatment and follow-up of PIH patients.

ACKNOWLEDGEMENT

We are thankful to Mr. Gener R. Ronquillo for typing the manuscript.

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