Evaluation of Oxidative Stress with Thyroid Function in Growth Hormone-deficient Children from Misan Government ,Iraq.

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ABSTRACT

Aim: Growth hormone deficiency (GHD) is a complex endocrine syndrome, which is most commonly known to affect the pediatric population. However, adults also suffer from GHD, and it is often seen as part and parcel of the aging phenomenon. We aimed to investigate the levels and relationships of antioxidants, lipid peroxidation and thyroid function with altogether in growth hormone deficiency .

Method:

This study was conducted to measure a group of hormones related with infected individuals with the shortage of the growth hormone by studying 57 sample Which represents patient groups were (38 boys and 19 girls) divided in to two groups according to age Group (1): consist of 26 patients (boys 14 and 12 girls) their aged ranged from 2 to 8 years and Group (2) Consisted of 31 patients (24 boys and 7 girls). Their ages ranged from 9 to 16 years in comparison with 50 samples taken from normal individuals as a control group. The concentration of hormones has been measured were human Growth hormone (hGH) Total Triidothyronin(TT3), Total Thyroxin (TT4),Free Thyroxin (FT4)Thyroid Stimulating (TSH), serum malondialdehyde (MDA) ,Vitamin C , Erythrocyte superoxide dismutase (SOD) activity, plasma Total antioxidant capacity (TAC)

Results: the children for growth hormone has been found decrease significantly in thyroid hormone as (T3. T4) while non significantly in (FT4) , and highly increased significantly in (TSH) level in patient compared with control group

To evaluate the blood oxidative as (MDA) and antioxidant status the MDA and TAC levels were significantly lower in patients group than in the control

group, . in addition , the SOD was non-significant, as for the vitamin C showed a significant highly change in the patients group compared with control group and this result was remarkable for the increase, where the results (5.61± 0.73 vs 3.89± 0.72) mg/dL.

Conclusions: This work has demonstrated that some parameters of the blood oxidative and antioxidant system are out of balance and relationship with thyroid function as thyroid hormone even impaired in GHD children should be taken into account.

Introduction

Human growth hormone (hGH) gene was located on chromosome 17q and which is considered one of five similar genes. Where only genes GH1 and GH2 (producing GHv) produce growth hormone with any systemic relevance [(1)]. The GH consists of 191 amino acid, single chain protein with two disulfide bridges. Though the primary function of GH, as its name would suggest, the promotion of longitudinal growth in childhood, it does however has wide-ranging action on muscle, bone and adipose tissue as well as a number of other organs of the body [(2).]

However, the term oxidative stress (OS) generally applied when oxidants out number antioxidants, when peroxidation products development and these phenomena cause pathological effects [(3).] Moreover, free radicals and other reactive species are possibility to play an important role in alary portions of human disease. Furthermore, the presence of serious imbalance between production of reactive species and the antioxidant predicative system belong to expanded production of reactive species alternately low levels of antioxidants prompts oxidative damage (OS) and improvement of different disorders [(4).]

So, the assessment of (OS) might make utilized as a nonspecific marker of systemic disorders in the human body, then if those standard medication indicates itself as not sufficient to decrease an progressing (OS), the applied therapy may require modification, for example, administration to organization about vitamins, antioxidants, and soon, we believe that some parameters of blood antioxidant status could be used for assessment for (OS) [(5).] It will be especially important to estimate OS in GHD children.

Furthermore, some workers reported an imbalance of the antioxidant parameters [6], where patients with adult GHD demonstrated a high degree of OS. However, some studies showed the relationships between GH secretion and thyroid hormones function as well as the effects of growth or treatment by administration on thyroid hormone levels have been the subject[6,5]. The thyroid gland secreted effective hormones metabolically regulated and biochemical process, as it has an important role in maintaining the level of metabolism in tissue. As well as, highlights the role of hormones in the thyroid that regulate cellular metabolism and growth development.

The well – publicized part of vitamin C as powerful antioxidant over the large number of functions it has in the body. It forms of ascorbic acid (ascorbate) and dehydroascorbic acid, vitamin C acts as cofactor or co substrate. The most important of vitamin C for the body growing and developing its function in collagen synthesis and repair collagen is the tough, fibrous intracellular material (protein) that is the principle component of skin and connective tissue, the organic substance of bones and teeth and ground substance between cell without collagen synthesis, the suitable is not possible [7]

Therefore, aim of this study was evaluated several parameters of the blood from oxidative as (MDA) and antioxidant system ; total antioxidant capacity (TAC) of plasma , activity of superoxide dismutase (SOD) and vitamin C, those relationship to hormonal test to study groups for the knowing the reason for lack of growth hormone and show the effect of the effective of the hormone such as thyroid gland hormone (T3, T4, TSH, FT4) and measurements of height and rate of body mass index (BMI).

Material and methods:

Distribution of patients with GHD according to all study groups.

The study was performed from December 2015 to July 2016 and carried out on the depend of local ethic committee of misan health administration for official approval , patients collected who attended the AL sadder hospital teaching and some private lab in masin city.

Study design

Total number of (107) children were distribution into two groups. The first group included individuals of growth hormone deficiency (patients group) involve (57 patients included; 38 boys and 19 girls). Divided in to two groups according to age,

Group (1)

26 patients (boys 14 and 12 girls) . Ages ranged from 2 to 8 years with mean age of 5.6± 2.8 years.

Group (2)

31 patients (24 boys and 7 girls). Ages ranged from 9 to 16 years with mean age of 4.71± 1.01 years

The second groups encompassing (control group) of 50 samples are also divided into age groups. Similar to the division of patient groups except for the difference in the number from age groups of (2-8) and (9-16) years included (27 boys and 23 girls).

The mean chronological age (CA) was 6.1±2.2 years; mean bone age (BA) was 2.6±0.9 years. None of them has ever undergone treatment with GH.

Blood samples were taken between 8:00 to 10:00 a.m. and 4:00 to 8:00 p.m after 12 hrs. of collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at 500 rpm for 15 min. The serum was then removed and stored at -20℃ until analysis.

1. **MiniVidas Automated Immunoassay Analyzer (Enzyme Linked Fluorescent Assay (ELFA):** for the detection of growth hormone and thyroid hormone as(T3,T4,freeT4 and TSH). The local research ethics committee approved the study protocol and informed consent was obtained from all participants.

2. **Oxidative status evaluation**

1-Serum MDA levels

1 mL of 0.6% thiobarbituric acid solution, 3 mL 1% phosphoric acid, and 0.5 mL of 10% tissue homogenate were put into a tube. This mixture was heated in boiled water for 45 min. An extract was obtained by adding 4 mL of n-butanol in cooled tubes and then MDA passing into the serum was measured using spectrophotometer at 532 nm wavelength. The serum MDA amount was measured in nmol/L.

2- Serum vitamin C levels

The concentration of vitamin C in serum was determined after collection according to a modified of method Washington and Toronto [8].

3. **Antioxidant status evaluation**

1-The erythrocyte SOD activity was estimated by inhibition of epinephrine self-oxidation at 25 ◦C. One unit of SOD activity was defined as amount of SOD required to cause 50% inhibition of the oxidation of the epinephrine. The SOD activity was expressed as units per gram of haemoglobin (Units/g Hb).

2-Estimate the total antioxidants capacity (TAC) in the blood plasma by the ferric reducing ability of plasma (FRAP) assay, with modifications: 350 µL of distilled water was added to the test tube containing 3 mL of the reagent (working solution),

Then 50 µL of plasma sample was added and mixed. After 10 min, the samples were read at a wavelength of 593 nm. This method is based on the reduction of colorless ferric (Fe^{3+}) tripyridyltriazine complex in working solution to blue colored ferrous (Fe^{2+}) tripyridyltriazine complex at low pH. The TAC values were obtained by comparing the absorption change in the test mixture with those obtained fromincreasing concentrations of Fe^{2+} and expressed as µmol of Fe^{2+} equivalents per liter of sample.

Photometric assay of the blood hemoglobin is based on the transformation of hemoglobin into its haemachrome form by sodium dodecyl sulphate, followed by absorption of the measuring light at 540 nm

Statistical methods:-paired t-test was used to compare the results of various parameters among the studied group. All values expressed as Mean±SD, and ($p \leq 0.05$) were considered to be statistically significant.

Results

Table (1) showed the division of the all study participants (patients and control group) as well as their division according to by age within groups

Table (1) Characteristics of all subjects group in this study

Study groups	Age(years)	No.	Sex	
			No. Boys	No. Girls
Patients group	2-8	26	14	12
	9-16	31	24	7
Control group	2-8	23	16	7
	9-16	27	11	16
Total		107	65	42

Table (2) decreased significantly in the growth hormone deficient (1.02± 0.22) ng/ml compared with control group (7.38± 1.57) ng/ml.

When comparing with the age groups was displayed a significant decrease in GH level in the age groups (2-8) , (9-16) years from patients whether they are boys or girls category as compared control groups ($p \leq 0.05$) (2.51± 1.39, 1.19± 0.92) respectively with control group was (5.82± 0.44 and 8.31± 0.59).

However, were found significantly decrease of thyroid hormone as (T3. T4) while none significantly in (FT4) level in patient groups. In addition, there is increased significantly in (TSH) level in-patient compared with control group. When comparing the in all thyroid hormone in our study between age groups indicate the presence of significant decrease in patient age group categories mentioned in the study that for patient who suffer from GHD table (3) compared with control group. Which refers to all the results had obtained ,table (2) it refers to both to evaluate the blood oxidative as (MDA) and antioxidant status (Vitamin

C, Superoxide dismutase (SOD) and total antioxidant capacity (TAC) levels. MDA and TAC levels were significantly lower in patients group than in the control group (0.99± 1.05 vs 8.62 ± 1.73 µmol/L and 277.6 ± 11.21 vs 918.3 ± 10.58 µmol/L) respectively .(p≤ 0.05) . In addition, the SOD was non-significant. (p≤ 0.05) in the patient group compared with control ,but it is attracting attention the presence of highly significant differences among age groups in the study.

Table 2: Effect of growth hormone value on all parameters of the GHD and the control

Parameters	Control	Patients	*P-value
Height cm	112.2± 6.21	81.6±10.3	0.021
Weight kg	20.5±1.3	15.4±3.90	0.002
GH.	7.38±1.57	1.02±0.22	0.015
T3	1.61±0.80	0.61±-0.19	0.032
T4	91.33±2.73	60.53±1.83	0.020
FT4	14.77±1.90	12.11±2.24	0.039
TSH	2.68±1.39	4.96±1.05	0.14
MDA	8.62±1.73	0.99±1.02	0.0051
Vit. C	3.89±0.72	5.61±0.73	0.05
SOD	13.9±2.81	16.81±4.27	0.006
TAC	918.3±10.58	277.6±11.21	0.005

* Highly significant at 95% level ($p \leq 0.05$, $p<0.01$) (values are mean ± SD),

Table 3:The effect of growth hormone on the parameters of the study based on the age groups in all study groups

Parameters	Control		Patients	
	2-8 years	9-16 years	2-8 years	9-16 years
Height cm	77.25±3.91	110.3±3.71	59.22±2.71	87.43±3.01
Weight kg	18.52±1.92	22.34±2.44	14.66±1.83	16.93±1.81
GH.	5.82±0.44	8.31±0.59	2.51±1.39	1.19±0.92
T3	1.27±0.51	1.31±0.20	0.92±0.33	0.81±0.47
T4	87.18±4.21	95.68±1.42	67.08±3.47	59.99±2.53

FT4	16.92±2.40	14.25±11.92	11.92±1.87	13.52±2.77
TSH	2.56±0.08	3.72±1.07	3.99±1.12	3.75±1.98
MDA	7.68±2.15	8.31±0.95	3.83±0.88	1.92±0.64
Vit C	4.80±1.29	3.27±1.75	5.11±0.39	5.64±1.77
SOD	11.45±3.61	12.19±1.55	17.82±1.49	16.72±2.48
TAC	720.3±11.24	561.3±7.68	221,8±12.09	181.4±17.39

*** Highly significant at 95% level ($p \leq 0.05$) (values are mean ± SD),**

Tough, vitamin C showed a significant highly change in the patients group compared with control group and this result was remarkable increase, where the results (5.61± 0.73 vs 3.89± 0.72) mg/dL

Discussion

The term hormone will be extended to include with chemicals produced by cells that effect on the same cells alternately the nearby cells. The disease is considered a hormonal disturbance where the hormone level will be increased or decreased, which may be affect the physical activity. The importance of growth hormone similar to important of hormone protein linked to growth hormone with special receptors the surface of the membrane target cell. The action of growth hormone to spread all over the place and exercise on the growth and metabolism of all tissue of the body to able the growth [9]. Where growth hormone deficiency occurs when the pituitary gland fails to produce sufficient level of the hormone because of inflammation or bleeding in to the gland itself.

In some children, the failure or low growth hormone may be accompanied via the failure of other pituitary hormone, in addition may be acquired as self of shock or trauma or injured at birth and later in childhood [10]. In other hand, malnutrition is one of the causes of delay in growth, as it is an important protein building on the level of cells and tissues to synthesis of many hormone.

Triiodothyronine T3 is an essential determinant of the normal postnatal somatic growth and skeletal development, and a vital regulator of bon and mineral metabolism in human [11]. Before puberty, thyroid hormone was appeared to be a major prerequisite for typical development of bone.

Moreover, thyroid hormone are among the essential direct biological controllers of growth plate and bone accretion. in addition , thyroid hormones

influence and associate with growth hormone (GH) , insulin – like growth factor – I (IGF – I) system and other hormone that controls stature and bone growth [12]. Through the scientific literature, the interrelation ships between the thyroid hormone faction and pituitary GH are complex and fully understood. the results of the present study was appeared that thyroid decreased in (T3, T4) whatever the any causes of thyroid function as hypothyroidism in child hold is almost invariably associated with growth failure in addition can be explained by the abnormal liver function prompting to decrease formation of combination of thyroid hormone bonded to globulin (TBG) – thyroid hormone binding globulin [13]. A study was carried out on a group from child of shorter status under the age 10 years compared with study group. It was observed a significant decrease in the concentration of the T3 hormone and showed the existence of appositive correlation between the T3 hormone and GH that was agreement with our results. Nevertheless, our explanation for this that the hormone. On thyroid hormone gland, an important role in bone growth as it stimulates the T3 to from bone through receptors on osteoblasts. As well as its impact on growth, line and maintain bone mass. In addition, the effect of thyroxine hormone as the decreasing lead to decreasing in the conformation building material of protein – T4 is important for building process of proteins [14]. The phenomenon of T4 and FT4 concentration decrease in GH deficient subject has been reported in several studies [15, 16]. Our results conform to previous observation concerning these problems. The most frequently quoted mechanism of changes in thyroids hormone levels especially (T3, T4) is GH mediated increase of peripheral T3 to T4 deiodination [17].

According to our observations , to the increased of TSH hormone in the serum of patients with GH deficient , there is an inverse relationship or negative retro spective between thyroid stimulating hormone and thyroid TSH as cause low levels T3,T4 in the serum to increase to the increase the concentration of TSH [18]. Progressions in TSH secretion during GH deficit are less evident than FT4 the recent wonder has been explained by an increase of somatostatin (being a natural TSH inhibitor) [19].

However, at the end of the debate, a hypothyroidism of childhood lead to discontinue, growth or deficiency and delayed age of bone defect in the formation of bone plate lets and short stature.

Anyhow, a few mechanisms might prompt of oxidative stress in GH decreased the most important one is the subnormal intake of nutrients such have proteins and vitamins, leading eventually to accumulation of ROSs. Moreover, reduce concentration of vitamin C as antioxidant anther effect on ROSs formation [20]. The second mechanism for oxidative stress may be anon – specific chronic activation of the immune system due to chronic inflammation [21]. Where increased ROS leads to disintegration of polyunsaturated fatty acids on the cell membrane and formation MDA. This process is called as lipid peroxidation [22]. Where present study was showed a significant decreased in MDA, erythrocyte SOD and TAC, which suggests that energy deficient state might result in enhanced lipid peroxidation and decreased antioxidant enzyme activities.

However, our explanation of these manifestations of results through the scientific literature shows that the alterations could be attributed to the insufficient intake of micronutrients antioxidant vitamins as (vitamin E, C). Concerning this paper have shown that so – called growth hormone does not an always exert a positive influence upon IGF- I production and therefore upon general body growth. Moreover, it appears that its somatic effects are strongly – dependent on thyroid hormone status and balance of oxidative – antioxidant. Therefore, it seems difficult to study growth hormone regulation and physiological effects without taking into account these parameters.

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تقييم حالة مضادات الأكسدة مع وظيفة الغدة الدرقية في الاطفال المصابين بنقص هرمون النمو في مدينة ميسان، العراق.

الخلفية والاهداف: يُفرز هورمون النمو (GH) من الغدة النخامية (Pituitary Gland) ولـه دور مهم جدًّا في تنظيم نمو الأطفال وفي عملية الأَيْض (Metabolism) وعمليات أَيْضِيَّة عديدة لدى البالغين

منهجية البحث

أجريت هذه الدراسة لقياس مجموعة من الهرمونات المتعلقة بالأشخاص المصابين بنقص هرمون النمو من خلال دراسة 57 عينة تمثل مجموعات المرضى (38 ذكور و 19 فتاة) مقسمة إلى مجموعتين حسب الفئة العمرية المجموعة(1): ويتكون من 26 مريضا(فتيان 14 و 12 فتاة) تراوحت أعمارهم بين 2 و 8

سنوات، ومجموعة(2) تتألف من 31 مريضا(24 ذكور و 7 فتيات) . تراوحت أعمارهم بين 9 و 16 سنة. بالمقارنة مع 50 عينة مأخوذة من الأفراد العاديين كمجموعة السيطرة. وقد تم قياس تركيز الهرمونات وهي كما يلي: human Growth Hormoe (hGH) ، Total Triidothyronine (TT3) ، Total Thyroxine (TT4) ، Thyroid Stimulating Hormone(TSH)) ،وبعض محددات التي تعمل على نظام الاكسدة البايلوجية وهي , Vitamin C ,serum malondialdehyde (MDA) Erythrocyte superoxide dismutase (SOD) activity, plasma Total antioxidant capacity (TAC)

النتائج:

اظهرت الدراسة انخفاضا معنويا في هرمون الغدة الدرقية (T3 T4) بينما لم يكن اي مؤشر معنويا في (FT4)، كما وجدت زيادة معنويه في قيمة TSH في الاطفال المصابين بنقص هرمون النمو مقارنة مع مجموعة السيطرة.

ومن ناحية لتقييم مضادات الاكسدة وهي MDA كانت مستوياتها و مستوى TAC أقل بكثير في مجموعة المرضى مقارنه مع المجموعة الضابطة. كما اظهرت نتائج الدراسة ان نسبة مستويات SOD في الدم كانت غير معنوية على العكس تماما من مستوى فيتامين C التي اظهرت تغيرا معنويا كبيرا في مجموعة المرضى مقارنة مع مجموعة السيطرة وكانت هذه النتيجة ملحوظة بلزيادة حيث اشيرت النتائج (5.61 ± 0.73 مقابل 3.89 ± 0.72) ملغ / ديسيلتر