Serum Oxidative Stress and Creatine Kinase Activity Related to Hoffman's Syndrome

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Abstruct

Several symptoms of hypothyroidism characterized by low FT3 and FT4 were classified into hypothyroidism and Hoffman's syndrome .It was accepted that free radical generation and lipid peroxidation are associated with many diseases. Creatine kinase in serum is affected by thyroid hormones. This study was undertaken to investigate the relationship between malondialdehyde as a general lipid peroxidation marker and CK activity in patients with primary hypothyroidism and Hoffman's syndrome which indicatees hypothyroidism with undetectable FT3 and FT4 in serum with elevated values of TSH.

The study revealed that MDA is within the normal range, while CK activities in sera of Hoffman's syndrome were increased with many folds compared to that of primary hypothyroidism and control values.

Introduction

Primary hypothyroidism is a chronic and insidious disease caused by a failure of thyroid hormone production. Hypothyroidism should be considered as a differential diagnosis of creatine kinase elevation ; actually, neuromuscular symptoms and signs occur in most newly diagnosed patients with thyroid diseases (1).

The frequency of myopathy in hypothyroidism ranges from 30-80%. The major symptoms related are weakness, muscular cramps and myalgia ,the pseudohypertrophic form is called Hoffman's syndrome(2) .Muscle hypertrophy is a rare occurrence in hypothyroidism. It is more commonly seen in males than females and

is referred as Hoffman's Syndrome in adults and Kocher-Debra-Semelaigne Syndrome in children(3).

A health cell condition is a measure of conversion of T4 into T3, which is catalyzed by 5-diiodinase (4), this is modified by the pituitary gland(5). Creatine kinase as a key of enzyme plays a significant role in the assessment of the thyroid function as a clinical score and the measurements of these peripheral thyroid hormones (6).

Alteration of basal metabolic rate and the energy metabolism of tissue in several mammalian species represents one of the major functions of thyroid hormones (7). Accumulating evidence has suggested that the hyperthyroidism is associated with the increase in free radical production and lipid peroxide levels (8,9), where as the hypo metabolic state induced by hypothyroidism is associated with a decrease in free radical production (10) and lipid peroxidation products (11).

Materials and Methods

Chemicals : All chemical used in this project were of high analar grade. CK-Kit from RANDOX lab. was used for the determination of CK activity.

ULRER a sensitive thyroid stimulating hormone U-TSH enzyme immunoassay test kit, free triiodothyronine FT3 enzyme immunoassay test kit and free thyroxin FT4 enzyme immunoassay test kit (Biocheck,Inc,Burligame).

Sampling:

The samples were collected from (The Specialized Centre of Endocrinology and Diabetes (Al-Kindi Hospital)

They were classified into three groups as the following:

1. Control Group(G1) : includes (14) healthy individuals from both sexes, with no previous disease which may interfere with the parameters analyzed in this study.
2. Hypothyroidism patient group (G2): includes (10) patients suffering from hypothyroidism of both sexes.
3. Hoffman's syndrome group (G3) : includes (3) patients suffering from hypertrophic muscular symptoms.

Collection of blood :

Blood samples (5 ml) were collected from the above groups by venipuncture, the blood was left at room temperature for 30-60

min. (no anticoagulant) after a clot formation , the serum was isolated by centrifugation at 2500 rpm for 10 min. .

Determination of serum malondialdehyde MDA :

Malondialdehyde is an end product of lipid peroxidation . It reacts with thiobarbeturic acid TBA to produce a colored complex . MDA was measured according to (12) the method of (Fong K.L.et.al.1973) by monitoring the TBA reactive complex formed in the incubation mixture containing plasma , TBA and trichloroacetic acid TCA in a water bath at 60 ^{o}C for 90 min .The absorbance of the complex was measured at 532 nm .

Determination of thyroid stimulating hormone TSH :

The ultra sensitive TSH- Elisa test is based on the principle of a solid phase enzyme linked immunosorbent assay (13,14).

Determination of free triiodothyronine FT3 and free thyroxin FT4 tests

The FT3 and FT4 tests are a solid phase competitive enzyme immunoassay (15).

Determination of CPK activity :

Creatine kinase CK activating was done by utilizing creatine phosphate as a substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH .

The NADPH produced is proportional to CK activity and is used to reduce nitro blue tetrazolium NBT in the presence of diaforase to give the blue /violet color of diaformazon which has a maximum absorption around 560 min, the reaction was stopped by the addition of hydrochloric acid (16).

Result and Discussion

Table(1.) and (fig. I) showed a marked elevation in TSH for G3 compared to G2 and G1, also TSH for G2 was elevated over that of control . While in table (1.) ,(fig. II) and(fig.III), FT3 and FT4 in G3 were found to be under the detectable limits .

Sub-clinical hypothyroidism SCH is characterized by the findings of the elevated TSH level in the presence of low T3 and T4 in the circulating system (17). The activity of the thyroid gland is predominantly regulated by the concentration of the pituitary glycoprotein hormone, thyroid stimulating hormone TSH (18).Severity of myopathy generally correlates with the duration and

the degree of thyroid hormone deficiency (19). Various physiological studies in both rats and humans confirmed the concept in that a decrease in T4 and T3 leads to an increase in TSH (20).

Table (2.) and(fig.IV) showed that MDA levels of hypothyroidism in G2&G3 were slightly lower than that of G1. Studies confirm that in hypothyroid rats, the MDA levels didn't differ significantly from euthyroid value (21).Thyroid hormones are physiological modulators of both tissue oxidative stress and protein degradation (22). The mechanism linking hypothyroidism with oxidative stress is unknown(23).

Table (2.) and(fig.V) showed an increase in creatine kinase by many folds in G3 compared to G1and G2 .Thyroid hormone increases the activities of many enzymes including CK .The mechanism of such elevation in CK in G3 may be related to increased leakage from skeletal muscle cells or diminished clearance from the circulation or both (24).Patients of G3 under score with assessing of thyroid function are suffering from weakness and muscle stiffness even when systemic symptoms and signs of hypothyroidism are moderate (25). Patient of G2 showed an increased levels of CK over that for control group . As hypothyroidism resulted in a deficiency of thyroid activity characterized by decrease in basal metabolic rate ,.

Fatigue and lethargy, these parameters were covering energy content . This process is prohibited by protein synthesis inhibitor (26).Most disorders are associated with protein biosynthesis for example severe hypothyroidism resulted in an elevation in CK activity (27). A study conduced to investigate CK activity and it's involvement in energy profile , the data obtained showed an elevation in CK activity in hypothyroidism when compared with control and the muscular disorders which leads to hypothyroid myopathy . (Hoffman's Syndrome) showed an elevated muscles enzyme even when compared with hypothyroidism (28) .

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Table(1): TSH, FT_3 and FT_4 values in sera of the three studied groups.

Groups	No.	TSH IU/ml Mean±S.D.	FT_3 Mean±S.D.	FT_4 Mean±S.D.
G_1	14	1.8±0.09	2.5±0.424	1.0±0.19
G_2	10	16.6±4.24	0.6±0.4	0.4±0.1
G_3	3	100±8.8	Undetectable (Under limit)	Undetectable (Under limlt)

Where: G_1 represents control group
G_2 represents group of patients with Hypothyroidism
G_3 represents group of patients with Hoffman's Syndrome

Table (2): MDA values and CK activities in sera of the three studied groups

Groups	No.	MDA nMole/L Mean±S.D.	CK IU/L Mean ±S.D.
G_1	14	11.02 ±1.489	110±16.2
G_2	10	9.3±1.25	1830±81.67
G_3	3	9.0±1.1	7320±52.67

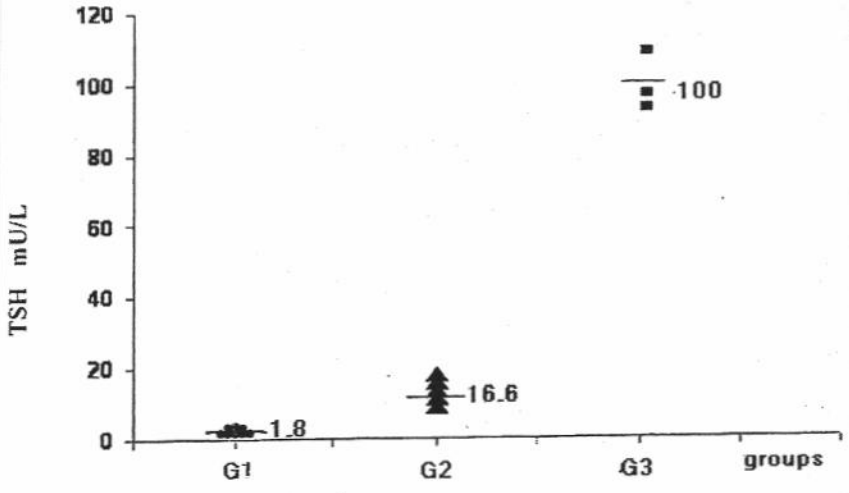


Fig I: TSH levels in the three studied groups

Where: • represents G_1 (control group).
▲ represents G_2 (Hypothyroidism patients group)
■ represents G_3 (Hoffman's syndrome patients group)

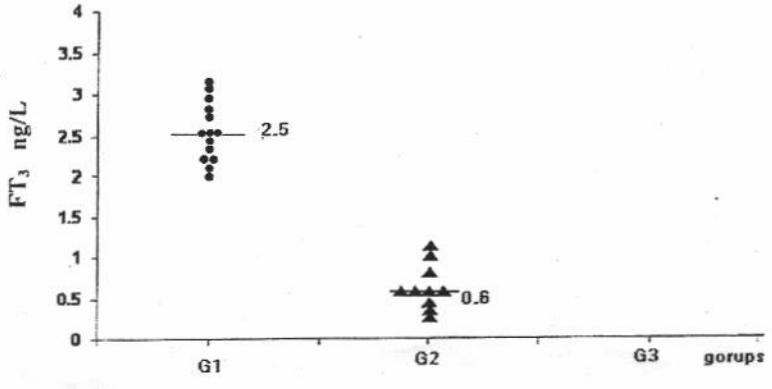


Fig (2) Levels of FT_3 in G_1 and G_2 group while in G_3 it is under limit

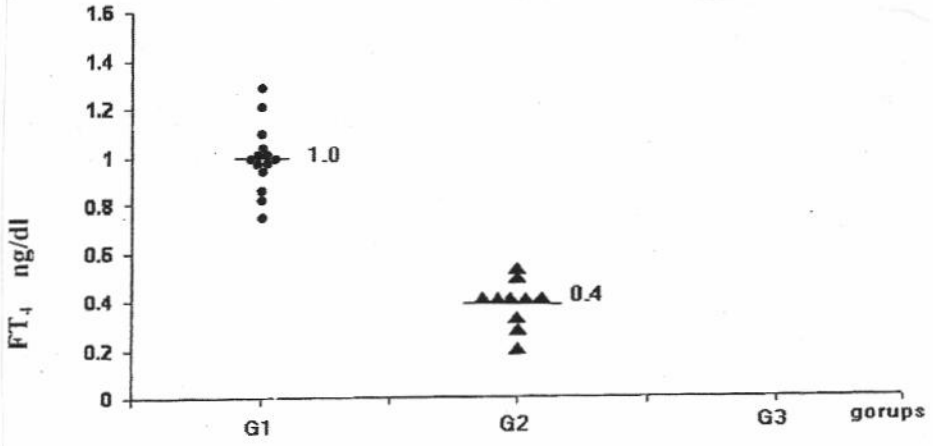


Fig (3): Levels of FT_4 in G_1 and G_2 while in G_3 it is undetectable

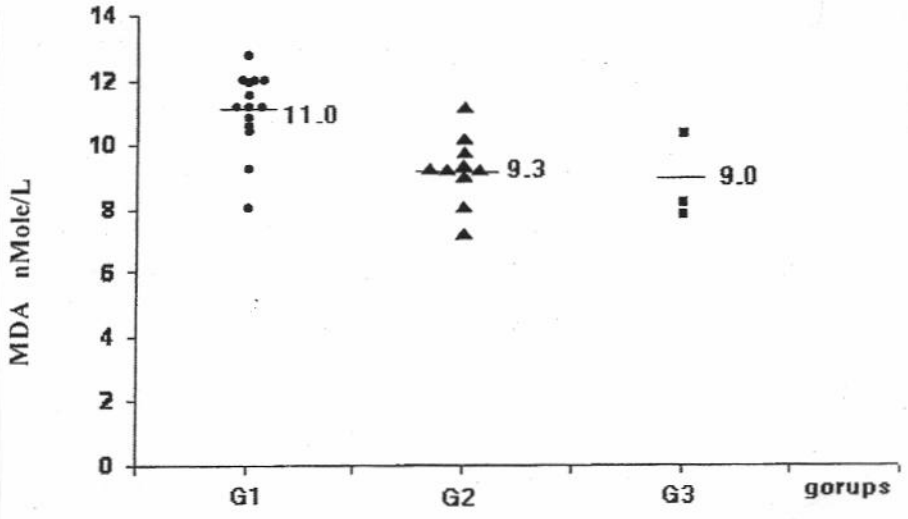
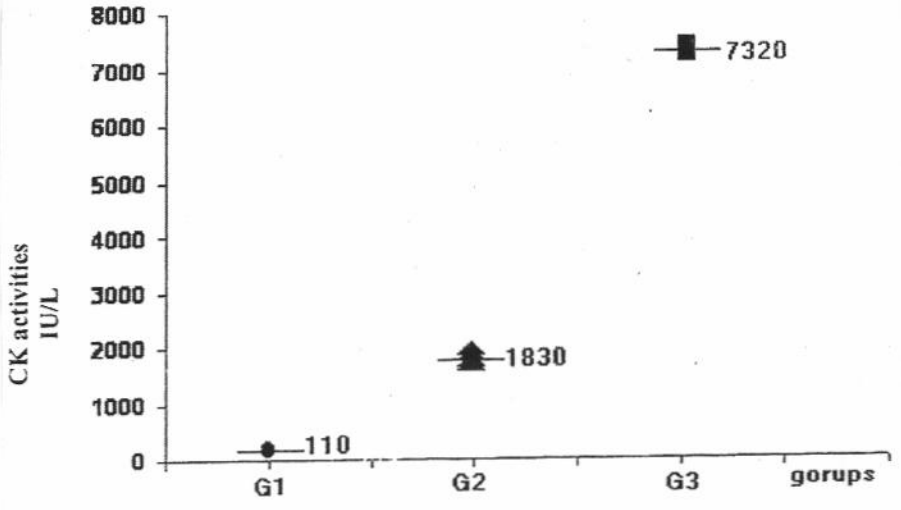


Fig (4): Levels of MDA in the three studied groups



Fig(5) Creatine kinase activities of the three studied groups

الشد التأكسدي وفعالية انزيم الكرياتين كاينيز في مصل المصابين بأعراض هوفمان

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الخلاصة

ان الاعراض التي تتميز بنقص هرموني الثايروكسين (FT4) وثلاثي أيودو الثايروكسين (FT3) ، تصنف ضمن مرضين هما قصور الغدة الدرقية ومرض اعراض هوفمان ، والاخير هو حالة نادرة من مرض قصور الغدة الدرقية ، كما أن فعالية أنزيم الكرياتين كاينيز (CK) في مصل الدم تتأثر بهرمونات الغدة الدرقية ولقد اصبح معروفا ان توليد الجذور الحرة والاكسدة الفوقية للدهون ترتبط بالعديد من الامراض .

ولهذا فان الهدف من هذه الدراسة هو ايجاد العلاقة بين المالون داي الديهايد وهو مؤشر الاكسدة الفوقية للدهون، وفعالية انزيم الكرياتين كاينيز في مرضى قصور الغدة الدرقية والمصابين باعراض هوفمان.

وقد تبين لدينا انه في بعض مرضى قصور الغدة الدرقية الذين تظهر لديهم اعراض هوفمان كانت مستويات هرمونات (FT3,FT4) في مصل الدم غير محسوسة ومصحوبة بارتفاع مستوى الهرمون المحفز للغدة الدرقية (TSH).

كما ان نتائجنا اظهرت ان قيمة المالون داي الديهايد (MDA) يقع ضمن الحدود الطبيعية، بينما تميزت فعاليت انزيم الكرياتين كاينيز في مصل الدم في المصابين باعراض هوفمان بزيادة حادة مضاعفة للاصحاء وحتى للمصابين بقصور الغدة الدرقية