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THE FUNCTION OF SIALIC ACID AS A RADICAL SCAVENGER IN EXPERIMENTAL HYPOTHYROIDISM WITH AND WITHOUT HYPERLIPIDEMIA

Hiperlipidemili ve hiperlipidemisiz deneysel hipotiroidizmde bir radikal tutucu olarak siyalik asidin etkinliği

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ABSTRACT

There is close relationship between hypothyroidism and oxidative stress. In hypothyroidism, lipid level, which is a potential target for oxidation, may be depressed by decreased free radical production. Our objective is to investigate the changes in sialic acid (SA) and oxidized-LDL (ox-LDL) levels in hypothyroid subjects with and without hyperlipidemia. Forty Wistar Albino rats were divided into 4 groups equally as follows: 1. Control group, 2. Hypothyroid group, 3. Hyperlipidemic group, and 4. Hyperlipidemic hypothyroid group. Thyroid and lipid parameters, ox-LDL and SA levels were determined in serum samples. Oxidized-LDL and SA were significantly higher in each group compared to the control group. Also hyperlipidemic hypothyroid group has increased lipid parameters, ox-LDL and SA levels compared to both hypothyroid and hyperlipidemic groups. Hypothyroidism accompanied by hyperlipidemia was observed to trigger the free radical oxidation. Changes in metabolic rate and increased lipid levels in serum may cause LDL oxidation, which in turn raises serum SA level. Serum SA level may be a marker of LDL oxidation.

Key words: Hypothyroidism, sialic acid, hiperlipidemia, LDL oxidation.

ÖZET

Hipotiroidizm ile oksidatif stress birbiriyle yakından ilgilidir. Hipotiroidizmde, oksidasyonun hedefi halindeki lipitlerin miktarındaki artış metabolizmanın baskılanması sebebiyle daha az radikal oluşumu beklenebilir. Amacımız hiperlipidemili ve hiperlipidemisiz hipotiroidili olguların ve kontrollerin siyalik asit ve oksitlenmiş LDL değişimlerini incelemektir. 40 adet Wistar Albino sıçan eşit olarak 4 gruba ayrıldı, gruplar: 1. Kontrol grubu, 2. Hipotiroid grubu, 3. Hiperlipidemik grup, 4. Hiperlipidemik hipotiroidi grubu. Alınan serum örneklerinde tiroid ve lipid parametreleri, okside LDL ve siyalik asit düzeylerine bakıldı. Kontrol grubuna kıyasla hem hipotiroidi ve hem hiperlipidemi gruplarında okside LDL ve siyalik asit seviyeleri artmıştır. Ayrıca, hiperlipidemik hipotiroidi grubunda, diğer gruplarla kıyaslandığında bu parametreler artmıştır. Hipotiroidiye eklenmiş hiperlipideminin, serbest radikal olusumunu tetiklediği gözlenmiştir. Metabolizma hızındaki değişiklik ve oksidasyonun hedefi olan serum lipid seviyelerindeki artış, LDL oksidasyonuna ve dolayısıyla serum siyalik asit seviyesinin artışına neden olabilir. Siyalik asit, LDL oksidasyonu icin bir belirteç olabilir.

Anahtar kelimeler: Hipotiroidizm, siyalik asit, hiperlipidemiya, LDL oksidasyonu.

INTRODUCTION

Thyroid hormones have effects on all steps of lipid metabolism, including lipid synthesis, mobilization and degradation, but lipid degradation is influenced more than the synthesis. Hypothyroidism is a condition in which serum thyroid hormone levels (triiodothyronine [T_3] and thyroxine [T_4]) are decreased. Thyroid stimulating hormone (TSH) is increased in primary hypothyroidism, where underfunctioning thyroid gland is the cause of hypothyroidism. Both basal metabolic rate and low-density lipoprotein (LDL) receptors in liver are reduced in hypothyroidism, which result in decreased fractional clearance of LDL. Total cholesterol levels, LDL and apolipoprotein B (apo B) levels are therefore increased in hypothyroidism. On the other hand high-density lipoprotein (HDL) levels are normal or even elevated in severe hypothyroidism because of decreased activity of cholesteryl-ester transfer protein and hepatic lipase, which are enzymes regulated by thyroid hormones (1,2).

Free radicals are reactive molecules with unpaired electrons that are produced as a result of normal cell metabolism. They are highly reactant and can participate in several cellular reactions, such as cell signaling, hormone synthesis and elimination of bacteria. Reactive oxygen species are a family of oxygen-containing chemical molecules that include both free oxygen radicals and non-free radical oxygen metabolites. The disturbance of antioxidant-oxidant balance and excessive production of free radicals increase ROS concentration, which can damage cell structures, a phenomenon known as oxidative stress. ROS mediated oxidative damage play a major role in pathogenesis of endothelial dysfunction and oxidation of lipids. Because of the decreased metabolic rate in hypothyroidism, free radical production is expected to be reduced, however increased levels of oxidative stress were also reported in previous studies (3-6). Additionally, excessive levels of TSH, which can be seen in primary hypothyroidism, was shown to cause increased production of oxidants (6).

Sialic acid (SA) is a N- or O- substituted derivative of neuraminic acid, and it is found as a terminal component of glycolipids and glycoproteins. SA has crucial biological functions, such as binding and transporting of positively charged compounds by its negative charge, being essential components of receptors and glycoprotein molecules. Also, SA can be used as a marker for several diseases (7-9). Excessive ROS can target oligosaccharides in protein structures and change their biological functions by breaking SAs located in the terminal position. SA was shown to eliminate H_2O_2 by acting as a H_2O_2 scavenger and converting it to H_2O (10,11).

Increase in lipid levels, which is a potential target of oxidation and oxidative stress, can cause LDL oxidation in hypothyroidism. On the other hand, a decrease in free radical production may be expected due to lower metabolic rate. Increased levels of free radicals cause both oxidation of LDL and desialilation of glycoproteins and glycolipids. In hypothyroidism, there is an increased tendency of free radical-related damage due to hyperlipidemia, but hypometabolic condition may have protective effects on free radicals. In the present study, we aimed to investigate the effect of hypothyroidism and hyperlipidemia on oxidized-LDL (ox-LDL) and SA levels in serum.

METHODS

Forty adult female Wistar Albino rats weighing 200-250 g were divided into 4 groups equally: 1. Control group, 2. Hypothyroid group, 3. Hyperlipidemic (HPL) group, 4. HPL hypothyroid group. Methimazole (75 mg/100 g diet) was added to the diet to create hypothyroidism in the second and forth group, and cholesterol (1,63 g %), cholic acid (0,41 g %) and sunflower oil (16,3 g %) were added to the diet of the third and forth group for 3 months in order to obtain experimental hyperlipidemia. The rats were maintained under stable conditions (23±1°C) and housed per cage on a 12h light/dark. At the end of the study, all animals were sacrified, blood samples were removed from the heart and collected into tubes. Serum samples were separated by centrifugation and used for biochemical analysis.

The study was approved by the Ethics Committee of the Marmara University.

Serum T_3, T_4, TSH and ox-LDL levels were measured by ELISA (catalog no: CSB-E05085r Cusabio, China; R6381 TSZ USA; R6394 TSZ USA, E90527Ra, USCN USA, briefly). Lipid levels were measured using commercial kits as follows: total cholesterol (catalog no:205 Biochemical Enterprise, Italy), total triglyceride (catalog no: TG383, Biochemical Enterprise, Italy), HDL (catalog no: HD321, Boichemical Enterprise, Italy) and LDL (catalog no: LDL348, Biochemical Enterprise, Italy).

Serum SA levels were determined using Warren's thiobarbituric acid method (12). The samples were incubated at 80°C for 1 hour in diluted sulfuric acid to liberate bound SA, then Warren's method was applied. This method consisted of oxidizing SA with periodate, terminated by addition of arsenite and then adding thiobarbituric acid. This resulted in the formation of a red-colored substance extracted in cyclohexanone. The absorbance of samples at 549 nm were read by spectrophotometer.

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego; CA; USA). ANOVA test was used for the comparison of groups and Kruskal Wallis test or Post-hoc Dunn test were used for post-hoc analysis.

Paired t-test (two sided) for normally distributed data and Mann-Whitney U test for nonparametric distributions were used to evaluate differences in the same group. Significance was defined as $p<0.05$ and the results are expressed as the means±standard deviation or as the median.

RESULTS

Serum T_3, T_4 and TSH levels of the groups are shown in Table 1. At the end of 3 months, serum T_3 and T_4 levels were significantly decreased and serum TSH level was significantly increased in the hypothyroid group compared to control group. Serum T_3 and T_4 levels were significantly decreased in HPL hypothyroid group compared to hypothyroid group and significantly decreased when compared with the HPL group. Serum TSH level was significantly increased in the HPL hypothyroid group when compared to the hypothyroid group and significantly increased when compared to the HPL group (Table 1).

Serum lipid parameters; total cholesterol, triglyceride, HDL and LDL are significantly increased in both hypothyroid group and HPL group relative to the control group. All lipid parameters were significantly increased in HPL hypothyroid group compared to hypothyroid group and HPL group (Table 2).

Table 1: Serum T_3, T_4 and TSH levels of the experimental groups.

Group	T_3 (ng/ml)	T_4 (ng/ml)	TSH (ng/ml)
Control	0,5 ± 0,25	19,80 ± 0,07	9,00 ± 0,03
Hypothyroid	0,18± 0,02***	10,60 ± 0,12***	14,00 ± 0,01***
HPL	0,51 ± 0,12	20,80 ± 1,27	8,60 ± 1,02
HPL hypothyroid	$0,11 \pm 0,01^{\delta,\Delta\Delta\Delta}$	$8,01 \pm 0,05^{\delta,\Delta\Delta\Delta}$	$20,00 \pm 0,08^{\delta,\Delta\Delta\Delta}$

$^{\delta}$ p<0,05 , ***, $^{\Delta\Delta\Delta}$ p<0,001

* significant according to control group

$^{\delta}$ significant according to HPL group

$^{\Delta}$ significant according to hypothyroid group

Table 2: Serum lipid parameters; total cholesterol, triglycerid, HDL, LDL levels of experimental groups.

Group	Total cholesterol (mg/dl)	Triglycerid (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	160,91±0,36	65,60±0,82	37,52±0,02	10,99±0,30
Hypothyroid	185,25±1,57*	80,70±0,02**	38 ,00±0,43*	15,00±0,31*
HPL	187,90±2,06***	93,95±0,30***	47,88±0,23***	28,57±0,02***
HPL hypothyroid	$214,70 \pm 0,49^{\delta\delta\delta,\ \Delta\Delta\Delta}$	$102,50 \pm 0,25^{\delta\delta,\ \Delta\Delta\Delta}$	$69,02 \pm 0,32^{\delta\delta,\ \Delta\Delta\Delta}$	$37,50 \pm 0,03^{\delta,\ \Delta\Delta\Delta}$

*,$^{\delta}$ p<0,05 , **, $^{\delta\delta}$ p<0,01 , ***, $^{\delta\delta\delta}$, $^{\Delta\Delta\Delta}$ p<0,001

* significant according to control group

$^{\delta}$ significant according to HPL group

$^{\Delta}$ significant according to hypothyroid group

Compared to the control group, serum ox-LDL levels in hypothyroid and HPL groups were significantly increased. Moreover, ox-LDL levels were significantly increased in HPL hypothyroid group compared to hypothyroid group and HPL group. SA levels were significantly increased in hypothyroid and HPL groups and compared to the control group and in the HPL hypothyroid group compared to both the hypothyroid and the HPL groups (Table 3).

DISCUSSION

Hypothyroidism is one of the most common causes of hyperlipidemia in humans and animals and it is characterized by excessive cholesterol and LDL levels. Hyperlipidemia, especially hypercholesterolemia is the most important risk factor for coronary artery disease and increased LDL is the most common lipid abnormality in hypothyroidism since lipids are substrates for lipid peroxidation. There are various factors of increased oxidative stress in hypothyroidism, such as hyperlipidemia, deficient or imbalanced antioxidant system and excessive TSH

(6). LDL oxidation plays a crucial role in atherogenesis by damaging the structure of endothelium and triggering endothelial dysfunction by entry ox-LDL to general circulation.

Table 3: Serum ox-LDL and SA parameters of experimental groups.

Group	ox-LDL (ng/dl)	Serum SA (mg/dl)
Control	6,43±0,14	70,04±12,82
Hypothyroid	8,58±0,32*	121,30±15,91***
HPL	8,37± 0,17***	113,30±8,53***
HPL hypothyroid	$10,23\pm0,10^{\delta\delta\delta,\Delta\Delta\Delta}$	$194,36\pm9,25^{\delta\delta,\Delta\Delta}$

* $p<0,05$, $^{\delta\delta}$ $p<0,01$, ***, $^{\delta\delta\delta,\ \Delta\Delta\Delta}$ $p<0,001$

* significant according to control group

$^{\delta}$ significant according to HPL group

$^{\Delta}$ significant according to hypothyroid group

In this study, all serum lipid parameters we investigated were increased significantly in the hypothyroid group compared to the control group. Also, in the HPL hypothyroid group all serum lipid parameters were significantly increased compared to the HPL group. Constantini et al reported that in hypothyroidism, LDL oxidation was strongly influenced by total cholesterol and serum LDL levels. In addition, hyperlipidemic subjects were shown to have significantly higher LDL oxidation than control subjects (5). Increasing the level of LDL cholesterol raises LDL oxidation, and formation of ox-LDL raises the risk of atherosclerosis. Cholesterolemia is a common condition in hypothyroidism, therefore hypothyroidism is a risk factor for atherosclerosis due to LDL oxidation (5,13). Also it was reported that excess TSH levels can cause over-production of oxidants in body and enhanced oxidative stress parameters were found in hypothyroidism (3-6). Our findings support the idea that hypothyroid patients have increased susceptibility to oxidative stress and LDL oxidation. SA increases rapidly following the injury or inflammatory process. Increased SA was shown to be a protective mechanism of the organism against free radicals (14-16). SA act as a H_2O_2 scavenger and eliminate excess H_2O_2 (11).

Desialylation of LDL has been shown to increase its binding to arterial proteoglycans and its uptake into cells (17-18). Also, a strong negative correlation has been reported between the SA content of LDL and the amount of cholesterol accumulated intracellularly (19-22). Excessive production of free radicals may cause desialylation of LDL by sialidase. Decreasing the SA content of LDL and increasing serum SA level via oxidative stress may reflect desialylation of LDL. Thus, the damage of the cell structure caused by oxidative stress may be a reason of increased sialidase activity and removal of SA from LDL. Once removed, it is secreted from cell into blood, increasing serum SA levels. Cerne et al (23) reported that decreased SA content of LDL was associated with an increased oxidized LDL. Consistent with these results, in our study, serum total sialic acid levels were increased significantly in all experimental groups according to their controls.

In conclusion, to our knowledge, this is the first study to report SA levels in hypothyroidism with hyperlipidemia in rats. Our findings suggest that, as seen in hypothyroidism and hyperlipidemia, increased lipid levels may lead to higher tendency to oxidative stress. Hyperlipidemic hypothyroidism can cause an increased sensitivity to oxidation and oxidative stress. Additionally, serum SA concentration may be a valuable indicator of LDL oxidation and it can be used to evaluate the lipoprotein oxidation.

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