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Evaluation of oxidant stress in Wilson's disease and non-Wilsonian chronic liver disease in childhood

Çocukluk çağında Wilson Hastalığı ve Wilson dışı kronik karaciğer hastalığında oksidan stresin değerlendirilmesi

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Background/aims: *Oxygen free radicals have an important role in the pathogenesis of acute and chronic liver disease. Free radical formation and oxidative damage, probably mediated with copper accumulation, are important in Wilson's disease pathogenesis. This study was performed to determine if accumulating copper in Wilson's disease is a cause of further oxidant stress compared to non-Wilsonian liver disease.* ***Methods:*** *In this study, we investigated plasma malondialdehyde and nitric oxide levels to estimate the oxidant stess and total antioxidant capacity and vitamin E/cholesterol, vitamin C and β-carotene levels to estimate the antioxidant status of patients. The groups investigated included 24 patients with Wilson's disease (group I), 25 patients with non-Wilsonian chronic liver disease (group II) and 23 healthy controls (group III). Wilson's disease and non-Wilson's disease patients were divided into subgroups according to disease stage (i.e. chronic hepatitis and cirrhosis) and all parameters were compared between subgroups and controls.* ***Results:*** *Malondialdehyde and nitric oxide levels were higher than controls in groups I and II (p=0.013, p=0.01), but these levels did not differ between the Wilson's disease and non-Wilson's disease groups. The parameters were also evaluated with respect to the disease stage (i.e. chronic hepatitis and cirrhosis), and there was no difference between groups I and II. Although malondialdehyde and nitric oxide levels were significantly different between both disease stage groups and the controls, we observed decreased vitamin C and β-carotene levels only in cirrhosis stage (p=0.01, p=0.01).* ***Conclusions:*** *We observed the presence of oxidant stress unrelated to the etiology of the liver disorder in our study. Deficiency of the major antioxidants, vitamin C and β-carotene, develops as the disease stage advances from chronic hepatitis to cirrhosis.*

Key words: Malondialdehyde, nitric oxide, total antioxidant capacity, antioxidant vitamins, Wilson's disease, children

Amaç: *Akut ve kronik karaciğer hastalığının patogenezinde serbest oksijen radikallerinin önemli rolü vardır. Wilson hastalığının patogenezinde biriken bakır aracılığı ile oluşan oksidatif hasarın rolü önemlidir. Bu çalışmada Wilson hastalığında biriken bakırın wilson dışı kronik karaciğer hastalıklarında oluşan oksidatif strese ek bir oksidatif stres oluşturup oluşturmadığı araştırılmıştır.* ***Yöntem:*** *Bu çalışmaya çocukluk yaş grubunda 24 Wilson hastası (grup I), 25 Wilson dışı kronik karaciğer hastası (grup II) ve 23 sağlıklı çocuktan oluşan kontrol grubu alınmıştır (grup III). Tüm gruplarda oksidan stresin değerlendirilmesi amacı ile plazma malondialdehit ve nitrik oksit, antioksidan durumu değerlendirmek amacı ile total antioksidan kapasite, vitamin C, beta karoten ve vitamin E/kolesterol oranı ölçülmüştür. Karaciğer hastaları hastalıklarının hepatit veya siroz evresinde oluşuna göre yeniden gruplanarak aynı parametreler açısından karşılaştırılmışlardır.* ***Bulgular:*** *Malondialdehit ve nitrik oksit düzeyleri grup I ve II de sağlıklı kontrollerden anlamlı oranda yüksek bulundu. Grup I ve II de kronik hepatit ve siroz olguları arasında oksidan stres ve antioksidan durum göstergeleri açısından fark saptanmadı. Malondialdehit ve nitrik oksit düzeyleri heriki hastalık grubunun heriki evresinde de kontrol grubundan yüksek bulundu. Vitamin C ve beta karoten düzeyleri ise sadece siroz evresinde kontrol grubundan anlamlı oranda düşüklük gösterdi.* ***Sonuç:*** *Bu sonuçlarla çocukluk çağı kronik karaciğer hastalığının patogenezinde etyolojiden bağımsız olarak oksidatif stresin rol oynadığı ve anti-oksidan vitaminlerden özellikle C vitamini ve beta karotenin hastalığın siroz evresinde düşüş gösterdiği belirlenmiştir.*

Anahtar kelimeler: Malondialdehit, nitrik oksit, total antioksidan kapasite, antioksidan vitaminler, Wilson hastalığı, çocuk

INTRODUCTION

Free radicals can be defined as molecules containing a single unpaired electron in atomic or molecular orbits. These molecules have an important role in the pathogenesis of tissue damage in various disorders. Oxidative stress results from any significant change of the ratio between the formation of free radicals and antioxidant defenses in the various tissues and organs. It is essential to al-

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so evaluate the antioxidant levels when exploring the role of free radical-mediated damage in various diseases (1-3).

The involvement of oxidative stress in the pathogenesis of liver injury has been investigated for many years (4-6). Wilson's disease (WD), a copper overload disease, is inherited as an autosomal recessive trait (7). While it is a relatively rare disorder in European countries, it is a widespread cause of chronic liver disease in childhood in Turkey, where consanguineous marriages occur in 25% of the population (8).

Trace elements including copper play an important role in many systems that have evolved to deal with free radicals (9). Oxidative stress resulting from an increased production of free radicals via the copper accumulation and defects in antioxidant defenses may be central to the toxic processes in WD (10-12).

The objective of this study was to determine if accumulating copper in WD is a cause of further oxidant damage compared to non-Wilsonian (non-WD) liver disease.

MATERIALS AND METHODS

The study included a total of 72 children: 24 with Wilson's disease (group I), 25 with non-Wilsonian chronic liver disease (group II) and 23 healthy controls (group III). The demographic characteristics of the groups are presented in (Table 1).

Table 1. Demographic characteristics of patients

	Wilson's Disease Group I	Non-Wilsonian Chronic Liver Disease Group II	Healthy Controls Group III
N	24	25	23
Gender(F/M)	11/13	13/12	11/12
Age (year)	11.7 ± 5.1	11.8 ± 3.53	10.17 ± 2.97

The diagnosis of WD was based on low ceruloplasmin (normal: >20 mg/dl), increased urinary copper excretion (normal: <50 μg/dl), characteristic histology in liver biopsy and elevated hepatic copper content (normal: <50 μg/g), presence of Kayser-Fleischer rings (in 11 patients) and by ruling out other possible liver disorders.

There were 15 cirrhosis (group Ia) and 9 chronic hepatitis (group Ib) cases in children with WD. The non-Wilsonian group consisted of 15 chronic B hepatitis and 8 autoimmune hepatitis cases. The diagnosis of autoimmune hepatitis was established by ruling out other possible liver disorders and determining autoantibody positivities. This group consisted of 15 cirrhosis (group IIa) and 11 chronic hepatitis (group IIb).

Blood samples were collected from all groups between 8:00 and 9:00 a.m. after an overnight fast. All subjects and their parents gave their informed consent to blood sampling for the study. Vitamin C level was immediately studied and the samples were kept at –70°C for the determination of β-carotene, vitamin E, total antioxidant capacity (TAOC), nitric oxide (NO) and malondialdehyde (MDA) levels.

The concentration of NO was determined with Griess reaction as described previously (13). MDA determination was performed by thiobarbituric acid reacting substance (TBARS) technique (14). TAOC was measured using the Trolox equivalent antioxidant capacity (TEAC) (15). Methods of analysis were Rindi's spectrofluorometric method for vitamin E, McCormick for vitamin C and Neeld-Pearson for β-carotene (16, 17, 18). In order to achieve a more accurate vitamin E plasma status, vitamin E/cholesterol ratio was used (19).

One-way ANOVA with Student-Newman-Keuls testing was used to compare the three groups with

Table 2. Oxidant and antioxidant parameters compared between the three groups

	Wilson's Disease (n=24)	Non-Wilsonian Disease(n=25)	Healthy Controls (n=23)	p
β-Carotene (μg/dl)	125.32 ± 12.75	119.69 ± 53.11	152.49 ± 10.33	0.069
Vitamin C (mg/dl)	1.07 ± 0.10	1.05 ± 0.53	1.34 ± 0.10	0.056
Vitamin E/Cholesterol (mg/L / mg/L)	0.10 ± 0.01	0.11 ± 0.0049	0.11 ± 0.01	0.611
Total Antioxidant Capacity (mmol/L)	1.94± 0.43	1.15 ± 0.44	1.93 ± 0.10	0.079
MDA (nmol/ml)	4.63 ± 3.59	3.37 ± 1.68	2.37 ± 0.35*	0.013*
NO (μmol/L)	27.18 ± 4.14	21.54 ± 21.76	9.84± 1.01*	0.001*

One-way ANOVA with Student-Newman-Keuls testing was used to compare the three groups regarding oxidant and antioxidant parameters
A value of $p<0.05$ was considered significant

respect to oxidant and antioxidant parameters. All values of $p<0.05$ were considered significantly different.

RESULTS

Group I and group II showed no difference with respect to the levels of MDA, NO, TAOC, vitamin C, β-carotene and vitamin E/cholesterol. MDA and NO levels were significantly higher in groups I and II than in group III (Table 2).

Studied parameters showed no significant difference between group Ia and group IIa. Although vitamin levels and TAOC showed no difference between group Ia, group IIa and group III, group III showed lower MDA and NO levels (Table 3).

Vitamin levels, TAOC, MDA and NO levels showed no difference between group Ib and group IIb. Group Ib and group IIb had lower β-carotene and vitamin C and higher MDA and NO levels than group III.

Vitamin E/cholesterol ratios were similiar between the groups (Table 4).

DISCUSSION

Oxygen free radicals might play a role in the pathogenesis of tissue damage in many pathological conditions, including liver diseases, where antioxidant tissue systems are reduced. The main causes of liver disease in childhood are viral infections, genetic and metabolic disorders, and autoimmune and toxic hepatitis. Although there are many studies in the literature investigating the role of oxidant stress in liver diseases, few include WD in childhood (4-6). It is well known that persistent oxidant stress causes mutative effects on cell DNA and increases fibroblastic activity, leading to cirrhosis and carcinoma. Previous studies have demonstrated that MDA levels increase and antioxidant capacity decreases in acute and chronic hepatitis (5, 6, 20). Sokol et al. and Mansouri et al. expressed that mitochondrial lipid peroxidation takes place at varying levels in liver disorders independent of etiology, but it is more important in copper toxicity (21, 22). In our study, we determined the existence of oxidant stress in chronic liver disease. But since the NO and MDA levels of WD and non-WD patients were similar, we concluded that ac-

Table 3. Oxidant and antioxidant parameters in the hepatitis groups

	Wilson's Disease Hepatitis (n=9)	Non-Wilsonian Hepatitis (n=14)	Healthy Controls (n=23)	p
β-Carotene (μg/dl)	174.93 ± 18.93	132.97 ± 64.32	152.49 ± 10.33	0.196
Vitamin C (mg/dl)	1.25 ± 0.19	1.05 ± 0.61	1.34 ± 0.10	0.221
Vitamin E/cholesterol (mg/L / mg/L)	0.09 ± 0.01	0.11 ± 0.0064	0.11 ± 0.01	0.768
Total Antioxidant Capacity (mmol/L)	1.31± 0.19	1.18 ± 0.42	1.93 ± 0.10	0.301
MDA (nmol/ml)	4.38 ± 1.27	3.7 ± 1.79	2.37 ± 0.36*	0.041*
NO (μmol/L)	23.58 ± 4.89	13.21 ± 5.27	9.84± 1.01*	0.002*

One-way ANOVA with Student-Newman-Keuls testing was used to compare the three groups regarding oxidant and antioxidant parameters, according to disease stage. A value of $p<0.05$ was considered significant

Table 4. Oxidant and antioxidant parameters in the cirrhosis groups

	Wilson's Disease Cirrhosis (n=15)	Non-Wilsonian Cirrhosis (n=11)	Healthy Controls (n=23)	p
β-Carotene (μg/dl)	95.56 ± 11.67	102.79 ± 28.82	152.49 ± 10.33	0.001
Vitamin C (mg/dl)	0.89 ± 0.45	1.045 ± 0.44	1.34 ± 0.10	0.019
Vitamin E/cholesterol (mg/L / mg/L)	0.010 ± 0.01	0.10 ± 0.0017	0.11 ± 0.01	0.974
Total Antioxidant Capacity (mmol/L)	2.32± 0.67	1.10 ± 0.47	1.93 ± 0.10	0.242
MDA (nmol/ml)	4.77 ± 0.93	3.73 ± 0.61	2.37 ± 0.36*	0.042*
NO (μmol/L)	29.34 ± 5.39	32.16 ± 29.65	9.84± 1.01*	0.006*

One-way ANOVA with Student-Newman-Keuls testing was used to compare the three groups regarding oxidant and antioxidant parameters, according to disease stage. A value of $p<0.05$ was considered significant

cumulating copper in Wilson's disease may not constitute any further oxidant stress than that which is already present in chronic liver diseases.

The profile of antioxidants in biological fluids and tissues may be helpful in assessing oxidative stress in humans. Plasma antioxidant status is the result of the interaction of many different compound and systemic metabolic interactions. Thus, the overall antioxidant capacity may give more biologically relevant information than that obtained from measuring concentrations of individual antioxidants. When studying specific disease in humans, the measurement of the major plasma antioxidants instead of all scavenging molecules might be sufficient, but it is not recommended to study only a single antioxidant or total antioxidant status since the levels may be affected by dietary habits (23, 24). In our study, although TAOC levels were similiar in all groups, vitamin C and β-carotene levels were found to be lower in WD and non-WD patients than in healthy controls, especially in cirrhosis stage. This result suggests that measurement of TAOC might give a misleading impression of antioxidant defenses. In many studies investigating vitamin levels in chronic liver diseases, it has been demonstrated that β-carotene levels are significantly lower in cirrhosis cases (25). Newsome et al. proved that as the disease stage advances from chronic hepatitis to cirrhosis and hepatocellular cancer, β-carotene levels decrease progressively (26). There have been previous studies reporting that lipid peroxidation only occurs when vitamin C is used completely. In experimental studies, rats were separated into three groups given vitamin E, retinol and b-carotene-supported diets, respectively, in order to investigate vitamin C level. Elevated vitamin C levels were observed in the b-carotene group (27, 28). The decrease of these two vitamin levels in our study, especially in cirrhosis stage, can be explained by their synergistic interaction. Decreased plasma and tissue vitamin E levels have been reported in chronic liver disease, including WD (29, 30, 31). As vitamin E determination was not performed in liver tissue in our study, it is not possible to claim whether vitamin E levels decrease in response to oxidant stress.

In conclusion, the presence of oxidant stress without relevance to the etiology of the liver disorder has been established in our study. Deficiency of vitamin C and β-carotene develops as the disease stage advances from chronic hepatitis to cirrhosis. This finding suggests the use of antioxidant therapy in various chronic liver diseases. But there is not sufficient information on antioxidant use of vitamins in ongoing liver disorders, especially in children. A further level of analysis would be the antioxidant profile in liver tissue, which might serve as a better indicator of disease-related oxidative stress than the plasma profile.

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