ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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PROPIONYLCARNITINE AND FREE CARNITINE ARE NEW BIOMARKERS IN THE FOLLOW-UP PERIOD OF MUCOPOLYSACCARIDOSIS TO SCREEN OXIDATIVE STRESS

MUKOPOLİSAKKARİDOZ HASTALARININ TAKİP SÜRECİNDE OKSİDATİF STRESİ TARAMADA YENİ BİYOBELİRTEÇLER, PROPİONİLKARNİTİN VE SERBEST KARNİTİN

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Öz

Amaç
Mukopolisakkaridoz (MPS) hastalarında oksidatif stresi taramak için uygulanabilir ve kolay bir prosedür mevcut değildir. Bu çalışmada amaç, MPS'deki oksidatif belirteçlere göre serbest karnitin (SK) ve propiyonilkarnitinin (PK) antioksidatif özelliklerini göstermek ve hasta takibinde basit ve kolay bir yöntem olarak kullanmaktır.

Gereç ve Yöntem
Çalışmaya, 27 MPS hastasında ve 24 sağlıklı gönüllü dahil edilerek bu hastalarda SK ve PK, antioksidatif belirteç olarak tandem kütle spektroskopisi kullanılarak çalışıldı ve malondialdehit (MDA), ise oksidatif belirteç olarak kullanıldı.

Bulgular
MPS hastalarında, PK ve SK seviyeleri önemli ölçüde azalırken, MDA seviyeleri sağlıklı gönüllülere göre daha yüksek bulundu. Enzim yerine koyma tedavisi alan MPS hastaları ile tedavi edilmeyen MPS hastaları karşılaştırıldığında, gruplar arasında anlamlı bir fark saptanmadı. Çalışma, MDA'nın PK ile anlamlı oranda ters orantılı olduğu bulundu (r = –0.402, P = 0.003). PK'nin MDA ile anlamlı bir korelasyona sahip olması dikkat çekiciydi.

Sonuç
MPS hastalarının daha yüksek MDA düzeylerine ve daha düşük PK ve SK düzeylerine sahip olduğunu göstermiştir, buna göre MPS hastalarının oksidatif yönde bir dengesizliğe sahip olduğunu ortaya koymuştur. PK ve SK, MPS hastalarının takibinde uygulanabilir bir yöntem olabilir ve hastaların enzim yerine koyma ve/veya antioksidan ilaçların tedavilerinin cevabının gözlemek için yeni biyobelirteçler olarak kullanılabilir.

Anahtar Kelimeler: Antioksidasyon, MDA, Mukopolisakkaridoz, Propionilkarnitin, Serbest karnitin

Abstract

Objective
There is no applicable and easy procedure to screen oxidative stress in mucopolysaccaridosis (MPS)

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patients. The aim herein was to show the antioxidative properties of free carnitine (FC) and propionylcarnitine (PC) with respect to oxidative markers in MPS and use a simple and easy method in patient follow-up.

Material and Methods
FC and PC were studied as an antioxidative marker using tandem mass spectroscopy and malondialdehyde (MDA) was studied as an oxidative marker in 27 MPS patients and 24 healthy volunteers.

Results
While the PC and FC levels were significantly decreased, the MDA levels were higher in the MPS patients than in the healthy volunteers.When compared between the enzyme-treated MPS patients and untreated MPS patients, there were no significant differences between the groups. MDA was found to inversely correlated with PC (r =–0.402, P=0.003).It was noteworthy that PC had a significant correlation with MDA.

Conclusion
The findings revealed that the affected patients had higher MDA levels and lower PC and FC levels, indicating an imbalance through the oxidative side. An applicable method of FC and PC measurement could be used to screen patients, considering them as new antioxidative markers in the patient follow-up period for the response of enzyme replacement therapy and/or antioxidant drugs.

Keywords: Antioxidation, Free carnitine, MDA, Mucopolysaccaridosis, Propionylcarnitine

Introduction

Mucopolysaccaridosis (MPS) is a group of lysosomal storage diseases characterized by accumulation of glycosaminoglycans (GAGs). The accumulation of undegraded GAGs leads to lysosomal dysfunction that leads to a wide variety of clinical findings.

The pathogenesis of MPS could be that GAG accumulation initiates inflammatory responses through the activation of some inflammatory mediators, and then the chronic inflammatory processes progress to oxidative damage through free oxygen species, which damage cell membranes and enzymes, resulting in protein and lipid peroxidation (1,2).

L-carnitine, which is an important biological compound found naturally in all mammalian cells exists either as either free carnitine (FC) or as acylcarnitine. The transport of fatty acids from cytosol into the mitochondria for β acid oxidation is carried out by L carnitine (3,4). Carnitine also has many roles in the metabolism of amino acids, removal of toxic free acylmetabolites, and it also balances free and acyl CoA metabolites (5–8). Propionylcarnitine (PC), which is an ester of L-carnitine and propionate, plays an important role in the metabolism of both carbohydrates and lipids (9).

Recent studies have shown that PC exerted an inhibitory effect in various models of inflammation and free radical induced membrane damage. It has been shown that PC counteracts the vasoconstrictor effect of endotelin 1 and increases the vasodilator effect of endothelial nitric oxide (10,11). It was reported that the endothelial dysfunctions of hypertensive rats that were chronically treated with either L-carnitine or PC had been reversed (12). These findings were also associated with PC activity on antioxidative mechanisms by reducing superoxide dismutase and also endothelial nitric oxide synthase (13–16).

PC plays a major role in the inflammation by reducing the release of some proinflammatory cytokines (17).

Under a healthy state, reactive species are formed and then degraded in balanced physiological concentrations; however, in inflammatory situations, excess free radicals are produced and those free radicals result in oxidative damage to biomolecules. After inflammatory pathway activation, the overproduction of reactive oxygen species enhances the peroxidation of fatty acids in mitochondrial membranes. Peroxidation of mitochondrial phospholipid membrane leads to the formation of reactive aldehydes, such as malondialdehyde (MDA), which is able to react with proteins and DNA, and then increase the inflammatory processes (18).

Based on the importance of PC and FC, in this study, it was aimed to show the antioxidative properties of PC and FC in MPS, a chronically slowly progressive disease, due to oxidative stress. To our knowledge, this is the first study to evaluate the antioxidative effects of PC and FC in MPS patients.

Material And Methods

Ethical Approval
Ethical approval was obtained from Gazi University

Faculty of Medicine Clinical Research Ethics Committee (13.11.2017 / 545). Written informed consent to participate and publish was obtained from all individual participants or their relatives included in the study.

Patients and Control

The study was conducted as prospective cross sectional study between September 2017 and January 2019. Twenty-seven MPS patients were included in the study as the patient group. The main clinical symptoms of the patients were skeletal deformities, hepatosplenomegaly, coarse face, hirsutism, short stature, and motor and mental retardation. The MPS patients were diagnosed via either enzymatic or molecular analysis, or both. Chosen as control group were 24 healthy children who were screened for routine examination. Among 27 MPS patients, 15 patients had been treated with ERT for at least 1 year. The subtypes of the patients were given in Table 1. The patients were examined before each analysis and those with any suspicion of infection in either group were excluded from the study. Patients with leukocytosis and increased c reactive protein levels were excluded from the study. (The values above cut-off were determined as leukocytosis and high CRP. The values were as follows: 7,47 x10.e3/uL,5 mg/ L respectively).

The Body mass indexes (BMI) were calculated and BMI over 25 kg/m2 was determined as overweight. The overweight and obese MPS patients were not included in to the study to exclude the effect of oxidant factor that obesity lead to.

Blood Samples

Blood samples were taken from the patients and control group under sterile conditions. A blood drop was absorbed on Guthrie paper for tandem mass spectroscopy and plasma samples were taken from MPS and healthy individuals to measure MDA. Samples were dried at room temperature for 3 to 4 h, and then stored –20 °C until analysis. From the MPS patients, samples were obtained before enzyme replacement therapy (ERT) while assessing vascular access. For the control group, samples were taken before the routine test analysis.

Determination of the Antioxidant Status

FC and PC were studied as antioxidative markers via tandem mass spectroscopy. A Waters Micromass Quattro Ultima (Waters, Milford, MA) tandem quadrupole MS/MS system equipped with an electrospray ion source (ESI) operating in positive mode was used for the analysis. The multiple reaction monitoring (MRM) mode was used to scan for specific mass ion intensities. Ions at m/z 85 produced by fragmentation were monitored. Concentrations of acylcarnitines (PC and FC) were measured by integrating the peak area and fitting with calibration curves using QuanLynx software (Waters, Version 4.1) CV of FC and PC were 17,19%, recovery 92 % (19).

Determination of MDA

The MDA levels were measured in plasma by the reaction of thiobarbutiric acid with colorimetric detection (Shimadzu UV-1601 Japan). Plasma samples were precipitated with trichloroacetic acid and mixed reactive thiobarbutiric acid. The mixture was incubated in a 95 °C water bath for 60 minutes, then rapidly cooled under water. The red complex formed was extracted in n-butanol. Absorbance was measured at 532 nm. As an external standard, tetramethoxy-propane was used, and the lipid peroxide level was expressed as nmol/ml MDA (20).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows 22.0 (IBM Corp., Armonk, NY, USA). The variables were investigated using visual (histogram, probability plots) and analytical methods with the Kolmogrov-Smirnov and Shapiro-Wilk tests to determine whether they were normally distributed or not. Descriptive analyses were presented as the mean and standard deviation (SD) for the normally distributed variables. MDA, PC, FC, and body mass index (BMI) were distributed normally, and the student t-test was used to compare these parameters between the MPS patients and control group. $p \leq 0.05$ was considered statistically significant. While investigating the associations between the non-normally distributed variables, the correlation coefficient and its significance were calculated using theSpearman correlation coefficient test.

Results

Individuals Included in the Study

There were 27 MPS patients who fell under different subtypes (Table I)

Among them 12 patients were females and 15 patients were males. The ages of the MPS patients ranged between 4,1 and 9,5 years old (Mean ± Standart deviation were 5,94± 1,85). Among fifteen patients with ERT treated, 7 patients were female and 8 patients were male. The ages were 5,96±2,06 year (mean±standard deviation). There were 5 females and 7 males in the ERT-untreated group. The ages

were 5,93±1,93 year (mean±standard deviation).

The healthy individuals were 11 females and 13 males whose ages were between 1 and 9,2 years old (Mean ± Standart deviation were 6,26± 2,12). There was no significant differences between ages (p=0,57 BMI were calculated and found that there was no significant differences between two groups (p=0,07).

Demographic features of the patients and individuals were shown in Table I.

Comparison of the MDA and Carnitine Levels Between the MPS Patients and the Control Group
To reveal the antioxidant status of the MPS patients and control group, it was found that the FC levels were decreased in the MPS patients. In the control group, the FC levels were higher than those in the MPS patients and there was statistical significance between the groups in terms of the FC ($p \leq 0.05$) (Table II).

Antioxidant status was then evaluated using PC, which plays major role in the antiinflammatory process. It was observed that the PC levels were lower in the MPS patients than those in the control group. When the PC levels were compared within the groups, significantly decreased levels were found in the MPS patients ($p \leq 0.05$). (Table II).
The MDA levels were studied to evaluate the oxidative status of the MPS patients. MDA levels were increased in the MPS patients, and as expected. In the control group, the MDA levels were much lower than those in the MPS patients. When the levels were compared, the differences between the groups were significant ($p \leq 0.05$) (Table II).

Comparison of the MDA And Carnitine Levels Between The ERT-Treated And Untreated Groups
In the ERT-treated patients, the FC levels were higher than those in the untreated group and no significance was found between the groups ($p>0.05$) (Table III).

PC levels were much higher in the ERT-treated group than in the untreated group. However, there was no significant difference between the groups ($p>0.05$) (Table III).

Although the MDA levels were decreased in the ERT-treated group and increased in the untreated group, no significant differences were observed between the groups ($p>0.05$) (Table III).

Correlation of MDA and Carnitine Levels Between MPS and the Control Group
When the MDA and FC were correlated in whole participants, it was found that there was no correlation between groups ($p>0.05$). It was noteworthy that PC had an inversely significant correlation with MDA ($p \leq 0.05$). Beside, for each group within itself, we could not find any correlation with FC, PC and MDA in MPS group in control group ($p>0.05$) (Table IV).

Table 1 The demographic features of the individuals

Groups	Number	Age (years)	Gender	BMI (kg/m2)
MPS patients	27	4,1-9,5	Male:15 Female:12	21-24,8
ERT treated group **MPS I** **MPS II** **MPS IV** **MPS VI** **MPS VII**	 2 2 3 7 1	4,5-8,7	Male: 8 Female:7	
ERT untreated group **MPS II** **MPS III** **MPS IV**	 1 9 2	2,7-9,5	Male:7 Female:5	
Healthy individuals	24	1-9,2	Male:13 Female:11	21,8-23,8

MPS: Mucopolysaccaridosis, BMI: Body mass index

Table 2 The laboratory evaluation of the individuals included in the study

Groups	MPS patients	Healty individuals	p values*[a]
C0 (mean ± SD) µmol/L	33,29± 10,14	39,48±9,44	0,029
C3(mean ± SD) µmol/L	1,02±0,80	2,05±89	0,000
MDA(mean ± SD) nmol/ml	7,45±2,61	3,78±0,75	0,000

MPS: mucopolysaccaridosis, C0: Free carnitine, C3: Propionyl carnitine, MDA: malondialdehyde, SD: standart deviation, * p ≤0,05 was significant a: Independent Samples t Test

Table 3 The comparison of free carnitine and propionylcarnitine in ERT treated and untreated patients

Groups	ERT-treated MPS patients	ERT-untreated	p values*[a]
C0 (mean ± SD) µmol/L	33.63 ± 10.57	32.86 ± 10.01	0.54
C3 (mean ± SD) µmol/L	1.25 ± 0.95	0.75 ± 0.46	0.21
MDA(mean ± SD) nmol/ml	7.08 ± 2.17	7.92 ± 3.10	0.94

MPS: mucopolysaccaridosis, C0: Free carnitine, C3: Propionyl carnitine, MDA: malondialdehyde, SD: standart deviation, * p ≤0,05 was significant a: Independent Samples t Test

Table 4 Correlation of free carnitine and propionyl carnitine with MDA

Groups	Antioxidant biomarkers	Oxidant biomarker	n	r	p*
In all groups	Free carnitine	MDA	51	-0.249	0.078
	Propionyl carnitine	MDA	51	–0.485	0.001
MPS patients	Free carnitine	MDA	27	-0,062	0,758
	Propionyl carnitine	MDA	27	-0,003	0,89
Healty individuals	Free carnitine	MDA	24	-0,14	0,50
	Propionyl carnitine	MDA	24	-0,47	0,83

n: Number, MDA: Malondialdehyde, * Spearman rho Correlation Test, * p ≤0,05 was significant

Discussion

The most important finding of this study was lower levels of PC and FC and higher levels of MDA in MPS patients, which might strongly suggest oxidative damage to lipids. Although the exact mechanism of increased oxidative stress and inflammatory pathway is not yet known, it is well known that lysosomes are very susceptible to any oxidative stress. After exposure to any oxidative events or agents,

antioxidative defenses attempt to reestablish the oxidant/antioxidant hemostasis; however, if reactive species begin to increase, the balance skips to the oxidative side. The importance of this study was that the measurement of PC and FC was a simple and applicable method to screen MPS patients with regards to oxidative stress. To our knowledge no studies exist that have evaluated the oxidative process via acylcarnitine analysis.

In some lysosomal storage diseases with a neurodegenerative course, oxidative damage has been studied. Pathological activation of neuronal cells after lysosomal deposition might trigger the release of oxidative and proinflammatory molecules. Several studies of some lysosomal storage diseases, such as metachromatic leukodistrophy, krabbe disease, Niemann-Pick type C, MPS I and MPS IIIB, and GM1 gangliosidosis have displayed oxidative cell damage in the disease pathogenesis (21–29). Jeyakumar et al. also showed that substrate reduction therapy decreased glycosphingolipid storage in Sandhoff mice by reducing microglial activation after oxidative damage and inflammation (26). Jeyakumar et al. showed that MDA levels were higher in the brains of Sandhoff mice due to induced oxidative damage resulting from lysosomal storage (26). Herein, it was verified that in MPS patients, MDA levels were higher than those in the healthy control group, suggesting that the patients had undergone an oxidative process and lipid peroxidation, indicating the same pathophysiology of other lysosomal storage diseases.

In an animal model of MPS I, superoxide dismutase and catalase activity were increased in the cerebellum, lungs, diaphragm, liver, and kidneys of MPS I mice (1). Filippon et al. suggested that in MPS II patients treated with ERT had lower levels of MDA than those of the untreated patients. They found that MDA was positively correlated with DNA damage and ERT was protective against oxidation (27). In this study, although MDA levels decreased in the ERT-treated group when compared with the untreated group, there was found no statistically significant difference between two groups. It could not support the previous studies. It might be attributed to the limited number of patients.

The antioxidative profile of PC has been evaluated in patients with chronic renal insufficiency and type 2 diabetes (28,29). The effective antioxidant activity of PC has been shown on the endothelial functions via MDA, 4-hydroxynonenal, and the nitrite/nitrate ratio. To date, there have been no studies evaluating the antioxidative properties of PC and FC in MPS patients.

In this study, it was suggested that MPS patients had low levels of PC and FC when compared with healthy subjects, which was supported by the fact that GAG accumulation had already started the inflammatory processes. PC levels were inversely correlated with MDA levels as PC had antiinflammatory functions which could be an indicator that PC had antioxidative properties. This might have been due to its propionate structure. There were 15 ERT-treated patients and 12 untreated patients in this study. The ERT-treated and untreated patients were compared to determine if the PC and FC levels were different between the groups. The FC and PC levels were higher in the ERT-treated group, while the MDA levels were lower when compared with the untreated group although we could not find any significance. These data might suggest that the accumulation of GAGs could induce the inflammatory pathway and oxidative process in MPS. Oxidative stress, which began with GAG deposition, might be prevented or reversed using ERT in MPS patients but due to limited number of patients, it could not find any significance results.

A limitation of this study was that it was performed on a small group of MPS patients. Studies with a larger group of subjects would be useful to evaluate the antioxidative properties of PC and FC. It might be beneficial to provide patients with antioxidant supplementation in addition to ERT to decrease inflammatory and oxidative processes.

Conclusion

In summary, oxidative parameters like MDA and antioxidant parameters, such as PC and FC, could not be balanced, which could have been the result of oxidative damage in MPS patients. Fluctuations in the FC and PC levels were evaluated for the first time herein, and it could be suggested that PC and FC levels might be used as a simple and applicable antioxidative biomarker in MPS patients, and they might show the effect of ERT on MPS patients.

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Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
Ethical approval was obtained from Gazi University Faculty of Medicine Clinical Research Ethics Committee (13.11.2017 / 545).

Consent to Participate and Publish

Written informed consent to participate and publish was obtained from all individual participants included in the study.

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References

1. Gustavo KA,Adalisa K, Marcos RO, Luisa MB, Melissa C. Alterations in Oxidative Markers in the Cerebellum and Peripheral Organs in MPS I Mice. Cell Mol Neurobiol 2009; 29: 443–448.
2. Pereira VG, Martins AM, Micheletti C, Almeida VD. Mutational and oxidative stress analysis in patients with mucopolysaccaridosis type 1 undergoing enzyme replacement theraphy. Clin Chim Acta 2007;387:75-79.
3. Bremer J. Carnitine: Metabolism and functions. Physiol Rev 1983;63(4):1420-1480
4. Bahl JJ, Bressler R. The pharmacology of carnitine. Annu Rev Pharmacol Toxicol 1987;27:257-277
5. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids 2004;70: 243-251.
6. Platell C,Kong SE, McCauley R, Hall JC. Branced –chain aminoacids. J Gastroenterol Hepatol 2000;15:706-717
7. Jogl G, Hsiao YS, Tong L. Structure and function of carnitine acyltransferases. Ann N Y Acad Sci 2004;1033;17-29.
8. Gan JLF, Simmons PA, Vehige J, Willcox MDP, Garrett Q. Role of carnitine in disease. Nutr Metab (Lond) 2010;7:30.
9. Malaguarnera M. Carnitine derivatives: clinical usefullness. Curr Opin Gastroenterol 2012;28:166-176.
10. Bertelli A, Giovannini L, Galmozzi G, Bertelli AA. Protective role of propionyl carnitine in vascular disorders experimentally induced by endothelin (ET-1) serotonin and K-carrageenin. Drugs Exp Clin Res 1993;7:7–11.
11. Bertelli A, Galmozzi G, Giovannini L, Mian M. Thrombosis induced by endothelin (ET-1) and carrageenin in rats treated with indomethacin and propionyl carnitine. Drugs Exp Clin Res 1993;19:75–78.
12. Bueno R, Alvarez de Sotomayor M, Perez-Guerrero C, Gómez-Amores L, Vazquez CM, Herrera MD. L-carnitine and propionyl-L-carnitine improve endothelial dysfunction in spontaneously hypertensive rats: different participation of NO and COX-products. Life Sci 2005;77:2082–2097
13. Bertelli A, Conte A, Ronca G, Segnini D, Yu G. Protective effect of propionyl carnitine against peroxidative damage to arterial endothelium membranes. Int J Tissue React 1991;13:41–43.
14. Bertelli A, Conte A, Palmieri L, Ronca G, Segnini D, Yu G. Effect of propionyl carnitine on energy charge and adenine nucleotide content of cardiac endothelial cells during hypoxia. Int J Tissue React 1991;13: 37–40
15. De Sotomayor MA, Mingorance C, Rodriguez-Rodriguez R, Marhuenda E, Herrera MD.L-carnitine and its propionate: improvement of endothelial function in SHR through superoxide dismutase-dependent mechanisms. Free Radic Res 2007;41:884–891.
16. De Sotomayor MA, Bueno R, Pérez-Guerrero C, Herrera MD. Effect of L-carnitine and propionyl-L-carnitine on endothelial function of small mesenteric arteries from SHR. J Vasc Res 2007; 44:354–364.
17. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–874.
18. Del Rio D, Stewart AJ, Pellegrini N.A review of recent studies on Mda as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005;15:316–328
19. Gucciardi A, Zaramella P, Costa I, Pirillo P, Nardo D, Naturale M., et al. Analysis and interpretation of acylcarnitine profiles in dried blood spot and plasma of preterm and full-term newborns. Pediatric Research 2015;77:36–47.
20. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem 2017;524:13-30
21. Hess B, Saftig P, Hartmann D, Coenen R, Lu ¨llmann-Rauch R, Goebel HH., et al. Phenotype of arylsulfatase Adeficient mice: relationship to human metachromatic leukodystrophy. Proc Natl Acad Sci USA 1996; 93:14821–26. doi:10.1073/pnas.93.25.14821
22. Wu YP, Matsuda J, Kubota A, Suzuki K. Infiltration of hematogenous lineage cells into the demyelinating central nervous system of twitcher mice. J Neuropathol Exp Neurol 2000;59:628–639
23. German DC, Liang CL, Song T, Yazdani U, Xie C, Dietschy JM. Neurodegeneration in the Niemann-Pick C mouse: glial involvement. Neuroscience 2002;109:437–50.doi:10.1016/S03064522(01)00517-6
24. Ohmi K, Greenberg DS, Rajavel KS, Ryazantsev S, Li HH, Neufeld EF. Activated microglia in cortex of mouse models of mucopolysaccharidosis I and IIIB. Proc Natl Acad Sci USA 2003;100 :1902–1907. doi:10.1073/pnas.252784899
25. Jeyakumar M, Thomas R, Elliot-Smith E, Smith DA, Van der Spoel AC, d'Azzo A et al. Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain 2003;126:974–987. doi:10.1093/brain/awg089.
26. Jeyakumar M, Smith DA, Williams IM, Borja MC, Neville DC, Butters TD, et al. NSAIDs increase survival in the Sandhoff disease mouse: synergy with N butyldeoxynojirimycin. Ann Neurol 2004;56:642–9. doi:10.1002/ ana.202429.
27. Filippona L, Wayhs CY, Atik DM, Manfredini V, Herber S, Carvalho CG, et al. DNA damage in leukocytes from pretreatment mucopolysaccharidosis type II patients; protective effect of enzyme replacement therapy. Mutation Research 2011;721:206–210
28. Signorelli SS, Fatuzzo P, Rapisarda F, Neri S, Ferrante M, Conti GO, et al. A randomised, controlled clinical trial evaluating changes in therapeutic efficacy and oxidative parameters after treatment with propionyl L-carnitine in patients with peripheral arterial disease requiring haemodialysis. Drugs Aging 2006;23(3):263-270
29. Santo SS, Sergio N, Luigi DP, Giuseppe Ma, Margherita F, Gea OA, et al.E ffect of PC on functional parameters and oxidative profile in type 2 diabetes-associated PAD. Diabetes Res Clin Pract 2006;72(3):231