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Serum Homocysteine and Lipoprotein(a) Levels in Preeclamptic Pregnants

Preeklamptik Gebelerde Serum Homosistein ve Lipoprotein(a) Düzeyleri

Murat Yaşar IŞILDAK, Sembol TÜRKMEN YILDIRMAK, Serkan DOĞAN, Mustafa ÇAKMAK,[2] Ekrem ÖZAKIN[1]

Departments of Medical Biochemistry and [1]Obstetrics and Gynecology, Okmeydanı Training and Research Hospital, İstanbul; [2]Department of Medical Biochemistry, Gülkent State Hospital, Isparta

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Objectives: The etiopathogenesis of preeclampsia has been shown to include the existence of vascular damage with endothelial dysfunction being the most directly related with development of the disorder. Elevated total homocysteine and lipoprotein(a) are risk factors for endothelial dysfunction and vascular diseases. We compared serum total homocysteine and lipoprotein(a) levels in healthy pregnant women and preeclamptics and investigated the relationship between these parameters.

Patients and Methods: Total homocysteine and lipoprotein(a) levels in serum of 28 women with preeclampsia and 25 normotensive women were measured by chemiluminescence and enzyme-linked immunosorbent assay methods, respectively. Both groups were composed of third trimester pregnants.

Results: Concentrations of total homocysteine (μmol/L) were significantly higher in women with preeclampsia than in normotensive pregnants. [8.54±4.76 vs. 4.85±1.50, (mean±standart deviation), respectively; p<0.001]. But no significant difference in lipoprotein(a) values (mg/dL) was observed in preeclamptics compared with matched healthy pregnants (Geometric mean: 4.15 mg/dL in preeclampsia vs. 2.30 mg/dL in controls).

Conclusion: There is a 1.8 fold increase in serum total homocysteine in preeclampsia compared to normotensive pregnants, which may cause vascular endothelial dysfunction. Circulating lipoprotein(a) is not significantly elevated in preeclamptics and thus is unlikely to play a role in the pathophysiology of this disorder.

Key words: Preeclampsia; homocysteine; lipoprotein(a).

Amaç: Preeklampsinin etyopatogenezinde, hastalığın gelişimi ile doğrudan ilişkili olan endotel disfonksiyonu ile birlikte vasküler hasarın varlığı gösterilmiştir. Yüksek total homosistein ve lipoprotein(a), endotelyal disfonksiyon ve vasküler hastalıklar için risk faktörleridir. Bu çalışmanın amacı sağlıklı ve preeklamptik gebe kadınlarda serum total homosistein ve lipoprotein(a) düzeylerini karşılaştırmak ve aralarındaki ilişkiyi araştırmaktır.

Hastalar ve Yöntemler: Yirmi sekiz preeklampsili ve 25 normotansif kadının serumunda total homosistein ve lipoprotein(a) düzeyleri sırası ile kemilüminesans ve enzim bağlı immünosorbent ölçüm yöntemleri ile tayin edildi. Her iki grup da 3. trimesterdeki gebelerden oluşturuldu.

Bulgular: Total homosistein konsantrasyonları (μmol/L) preeklampsili kadınlarda normotansif gebelerden anlamlı derecede yüksek bulundu [sırası ile, 8.54±4.76; 4.85±1.50, (ortalama±standart sapma); p<0.001]. Fakat preeklamptiklerde gözlenen lipoprotein(a) değerleri (mg/dL) sağlıklı gebelere kıyasla önemli derecede farklılık göstermedi (Geometrik ortalama: preeklamptiklerde, 4.15 mg/dL; kontrollerde 2.30 mg/dL).

Sonuç: Preeklampside serum total homosistein düzeyinde normotansif gebelere kıyasla 1.8 kat artış vardır. Bu artış vasküler endotelyal disfonksiyona yol açabilir. Dolaşımdaki lipoprotein(a) preeklamptiklerde önemli bir yükselme göstermediğinden, bu hastalığın patofizyolojisinde rol oynamadığı görülmektedir.

Anahtar sözcükler: Preeklampsi; homosistein, lipoprotein(a).

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Correspondence (İletişim adresi): Dr. Sembol Türkmen Yıldırmak. S.B. Okmeydanı Eğitim ve Araştırma Hastanesi, Tıbbi Biyokimya Kliniği, 34400 İstanbul. Tel: 0212 - 221 77 77 Fax (Faks): 0212 - 221 78 00 e-mail (e-posta): yildirmaksembol@gmail.com

Homocysteine (Hcy) is an amino acid containing sulphur which is formed during methionine metabolism. Remethylation of homocysteine to methionine and condensation with serine affect Hcy levels. Dysfunction of the enzymes or deficiency of cofactors in these reactions result in hyperhomocysteinemia. Accumulation of Hcy in tissues may cause free radical production during autooxidation of Hcy and so resulting in endothelial damage and dysfunction. Hcy thiolactone which is a high degree autooxidation product of Hcy, by combining low density lipoproteins (LDL), causes aggregations of LDL. Hcy thiolactone aggregates are taken up by intimal macrophages and foam cells are formed. Because of such effects of Hcy, atherosclerotic plague formation becomes easier. Hcy stimulates smooth muscle proliferation, inhibits growth of vascular endothelial cells and facilitates thrombin formation.

Lipoprotein(a) [Lp(a)] is a macromolecular complex which is formed by one or more apolipoprotein(a) molecules. It contains apolipoprotein B-100. Even though physiological function of Lp(a) has not been clearly understood, it is known as a genetically transmitted lipoprotein which is associated with increased atherosclerosis prevalence. The mechanism of increasing atherogenic risk of Lp(a) is complicated. It is presumed that the atherogenic potential of Lp(a) emanates from its LDL-like component and atherogenic cholesterol carrying capacity. It is considered that Lp(a) makes complexes with glycosaminoglycans or proteoglycans in atheromatous regions and/or goes under a serial of structural changes including chemical modifications with free oxygen radicals. Another way of increasing the risk of atherosclerosis of Lp(a) is the binding capacity to intima fibrin which may cause capturing of Lp(a) in atheromatous regions and resulting in growth of plaque. Apart from these, apo(a) has a similar structure as plasminogen. This situation indicates Lp(a) has a contribution to the etiology of atherosclerosis in prothrombotic phase too. Lp(a) inhibits plasmin which is activated by streptokinase and tissue plasminogen activator (t-PA) and competes with plasminogen to bind plasminogen receptors on the endothelial surface. Thereby, it is considered that it deteriorates intravascular thrombolysis and it initiates thrombogenic incidents on the endothelial surface potentially.

The cause of the endothelial cell injury in preeclampsia is multifactorial. In preeclampsia, characteristic pathological lesions in the placenta are fibrin deposits, acute atherosis and thrombosis. The similarity between the lesions of preeclampsia and atherosclerosis has led to speculations of a common pathophysiological pathway. Elevated plasma Lp(a) and Hcy concentrations are known risk factors for atherosclerotic cardiovascular disease.

Previous studies have produced contradictory findings regarding tHcy and Lp(a) levels in women with preeclampsia. There are some studies which report that total Hcy (tHcy) levels are increased in preeclampsia[1-5] but there is little information about the relationship between serum Lp(a) and tHcy levels in preeclampsia.[5] The aim of our study is to detect and compare tHcy and Lp(a) levels in pregnant women with normal blood pressure and preeclamptic pregnant patients, and to investigate the correlation between them and the roles of these parameters in etiopathogenesis of preeclampsia.

PATIENTS AND METHODS

Twenty-eight pregnant patients in the 3rd trimester having preeclampsia diagnosis composed patient group; 25 healthy pregnant women in 3rd trimester composed control group. Twelve in preeclampsia and eight in control group were primigravidas, and the rest were multigravidas. The gravidas who had a blood pressure of 140/90 mmHg or over measured twice at least in 6-hour intervals, having a urine protein level over 300 mg/day and the gravidas having edema +1 or over after 24-hour bed rest were accepted as preeclamptic. Subjects with any systemic disorder were excluded from the study. Since dietary factors may influence tHcy levels, the blood samples were obtained following a 12-hour fasting in both control and preeclampsia groups. The blood samples were taken in 10 mL vacuum tubes which did not include anticoagulants during resting position between 08.00-10.00

Table 1. Demographic, clinical and laboratory data in the groups§

	Control group (n=25)	Preeclamptic group (n=28)
Age (years)	27.84±5.80	28.75±5.43
Gestational age (week)	31.12±2.85	32.43±2.66
Blood pressure (mmHg)		
Systolic	109.60±7.90	147.14±7.63**
Diastolic	70.80±4.93	96.07± 8.32**
Parity		
Nulliparous(n)	8	12
Multiparous(n)	17	16
Gestational age range (week)	26-36	27-36
Lp(a) (mg/dL)		
Mean±SD	4.50±4.58	8.06±8.72
Geometric mean	2.30	4.15
tHcy(μmol/L)	4.85 ±1.50	8.54±4.76*

§: Compared with independent t-test; *p<0.001; **p<0.0001

am. Serum Lp(a) levels were detected by ELISA method in the analyzer ELx50- Elx800 (BioTek Instruments, Inc., Vermont, USA) using reagent Macra Lp(a) (Trinity Biotech, Ireland) and serum tHcy levels were detected by chemiluminescence method in the analyser DPC-Immulite 2000 (Siemens-DPC, Los Angeles, USA).

Statistical analysis

Statistical analyses were performed by GraphPad Prisma v.3 software. Independent t-test was used to compare two groups and Fisher authenticity test was used to compare qualitative data. The relative ratios of nulliparity under effect of tHcy were calculated (Odds Ratio=OR). Since it was observed that Lp(a) was not compatible with normal distribution, it was evaluated after logarithmic transformation and results were presented with geometrical mean. Accepting $p<0.05$ as significant, results were evaluated in 95% confidence interval. The Pearson test was used for correlation analysis.

RESULTS

In our study, there was no statistically significant difference in terms of age, gestational week and parity between preeclampsia and control groups and it indicates two groups are similar to each other in terms of definitive properties. THcy levels (μmol/L) ranged from 2.92 to 8.58 in the control group and 3.71 to 21.3 in the preeclamptic group. Mean tHcy concentration in preeclamptic patients was 8.54±4.76 μmol/L and the difference was significant when compared with controls (4.85±1.50 μmol/L, $p<0.001$) (Table 1). Geometric mean of Lp(a) in preec-

Table 2. Relative risk values according to receiver operating characteristic (ROC) curve

tHcy (μmol/L)	Sensitivity	Specificity	Positive cut-off value	Negative cut-off value	Accuracy	Relative risk
2-4	0.93	0.28	0.59	0.78	0.62	2.66
4-5	0.75	0.64	0.70	0.70	0.70	2.30
5-6	0.68	0.84	0.83	0.70	0.75	2.75
6-7	0.57	0.88	0.84	0.65	0.72	2.39
7-8	0.32	0.92	0.82	0.55	0.60	1.81
8-10	0.29	1.00	1.00	0.56	0.62	2.25
10-12	0.18	1.00	1.00	0.52	0.57	2.09

Table 3. The effect of nulliparity on preeclampsia

		Preeclampsia	Control
Nulliparous pregnants (=1)		n=12	n=8
Multiparous pregnants (>1)		n=16	n=17
Total		n=28	n=25
	p=0.59	Odds Ratio=1.6 (0.5-4.92)	

lamptic patients was 4.15 mg/dL and the difference was not significant when compared with controls (2.30 mg/dL) (Table 1). The correlations of serum of serum Lp(a) levels with age, gestational week and parity were studied but there was no statistically significant correlations between any of them. No significant correlation was found between tHcy and Lp(a) concentrations in the groups (r=0.155; p=0.267). There was a positive correlation between tHcy levels and only with gestational week ($p<0.05$). In order to determine the cut-off points of tHcy values of control and preeclampsia groups, receiver operating characteristic (ROC) curve was drawn and it was observed that after 6 μmol/L level the risk is 2.75 (Table 2). When the effect of nulliparity on preeclampsia was sought, although there was not any statistical significance, it was detected that nulliparity had a risk value of 1.6 (0.5-4.92) of increasing preeclampsia (without effect of tHcy) (Table 3). When tHcy levels were ≥6 μmol/L and the effect of nulliparity on preeclampsia was sought, although there was no statistical significance, it was detected that nulliparity (under effect of tHcy) had a risk value of 6.65 (0.31-141.03) increasing preeclampsia (Table 4).

Table 4. The effect of nulliparity and hyperhomocysteinemia on preeclampsia

		Preeclampsia (n=28)	Control (n=25)
tHcy (≥6 mmol/L)			
Nulliparous pregnants (=1)		n=8 (%28.5)	n=0 (%0)
Multiparous pregnants (>1)		n=11 (%39.2)	n=4 (%16)
Total		n=19 (%67.8)	n=4 (%16)
	p=0.28	Odds Ratio=6.65 (0.31-141.03)	

DISCUSSION

In our study, we observed that tHcy levels of preeclamptic patients were 1.8 times higher than those of control group. Laivuori et al.[1] and Rajkovic et al.[2] compared serum tHcy levels of preeclamptic and normotensive gravidas in 3rd trimester and they observed that tHcy levels were higher in preeclamptic group. Wang et al.[3] and Sanchez et al.[4] argued that hyperhomocysteinemia might be a risk marker for placental vascular disease and maternal preeclampsia. Vanderjagt et al.[5] reported that preeclampsia is associated with increased tHcy levels. Our findings are compatible with those of these authors. Ophir et al.[6] measured homocysteine concentration in serum of women who had preeclampsia during pregnancy and of women with noncomplicated pregnancy after delivery. They found that serum homocysteine levels were significantly higher in pregnancies with preeclampsia as compared with the uncomplicated pregnancy group. In contrary, Mayerhofer et al.[7] reported that they could not find statistically significant difference between groups of 45 preeclamptic and 45 normotensive gravidas in terms of tHcy levels. Both preeclamptic and normotensive groups were composed of gravidas who had a standard life and nutrition style, high levels of tHcy in preeclamptic group can not be attributed to any dietary factor. However;

i) There are studies which indicate that even in a minor renal deficiency, tHcy levels increase.[8] Due to renal functional changes observed in preeclampsia, serum tHcy levels may increase.

ii) Since fetal tHcy need is lower in preeclamptic gravidas, tHcy passing to umbilical vein decreases and so maternal serum tHcy levels may increase.[9]

We found that the risk of increasing preeclampsia of nulliparous gravidas having a tHcy value ≥6 μmol/L is 6.65 times higher than multiparous gravidas having a tHcy value <6 μmol/L. Although there was no statistical sig-

nificance, this result suggested that combination of hyperhomocysteinemia and nulliparity might play a role in the pathogenesis of preeclampsia.

The mechanism by which hyperhomocysteinemia participates in the pathogenesis of preeclampsia is complex and is still not understood completely. It may be mediated through oxidative stress mechanisms or through direct damage to the vascular endothelium. High tHcy levels may trigger many pathophysiological incidents such as degradation of endothelial nitric oxide production, vascular smooth muscle proliferation, coagulation anomalies and increase lipid peroxidation. High tHcy may be one of the various heterogenous diseases causing preeclampsia at the end.

Although endothelial cellular dysfunction is a critical step in the pathogenesis of preeclampsia, actually it is multifactorial. The characteristic lesion in uteroplacental region in preeclampsia is a necrotizing arteriopathy including fibrinoid necrosis, foam cell accumulation in decidua, fibroblast proliferation and perivascular infiltration (acute atherosis). After determination of strong relationship of abnormal lipid profile with atherosclerosis and endothelial dysfunction, the interest for lipid profile is gradually increasing in the studies involving pathogenesis of preeclampsia. In our study, we determined serum Lp(a) levels of preeclamptic and normotensive gravidas in 3rd trimester in addition to serum homocysteine levels. Since Lp(a) levels of groups were within a wide range, we evaluated geometrical mean instead of arithmetic mean and standard deviation. We determined the geometric mean of Lp(a) levels of preeclamptic group as 4.15 mg/dL and that of control group as 2.30 mg/dL. However, in contrary to serum tHcy levels, we could not detect any statistically significant difference between two groups in terms of serum Lp(a) levels. Wang et al.[10] made a study in 26 preeclamptic and 24 healthy gravidas and they observed that Lp(a) levels were higher in preeclamptic group compared to normal pregnancy; they noted that Lp(a) might serve as a marker of the pathogenic process of preeclampsia. Kamiński et al.[11] detected that levels of Lp(a) of 19 preeclamptic gravidas were higher than those of healthy gravidas and they claimed Lp(a) might be an important predictive factor in developing eclampsia. Djurovic et al.[12] measured serum Lp(a) levels of 157 preeclamptic gravidas by electroimmunoassay and radioimmunoassay and they detected that those were lower than those of 76 healthy gravidas. They reported that Lp(a) had a role in pathophysiology of preeclampsia. Bayhan et al.[13] found that serum Lp(a) levels were significantly higher in severely preeclamptic and mildly preeclamptic women than in normal pregnant women. They measured serum Lp(a) levels by a nephelometric agglutination assay method. Our findings were in contradiction with those of these authors.[10-13] In addition to these, several authors reported that they did not observe a statistically significant difference between serum levels of Lp(a) of preeclamptic and healthy gravidas.[14-20] Our findings were compatible with those of these authors. In determinations of serum Lp(a) levels, different methods were used as electroimmunoassay,[12] radioimmunoassay,[13] nephelometric agglutination assay,[13] double antibody ELISA[15] and turbidimetric immunoassay methods.[17,18]

There is little information about the relationship between serum Lp(a) and tHcy levels in preeclampsia.[5] In our study, no significant correlation was found between tHcy and Lp(a) concentrations in the groups. This finding is compatible with those of Vanderjagt et al.[5]

It seems that the results of studies on serum tHcy and Lp(a) during normal pregnancy and preeclampsia are very diverse. We think that the reasons originate from differences in the methods used, study design, sample sizes and ethnicity of study populations. In conclusion, no statistically significant difference existed between normotensive pregnant, preeclamptic women, in terms of plasma Lp(a) levels. This result suggests that circulating Lp(a) does not contribute significantly to the pathogenesis of preeclampsia. However, elevated maternal serum homocysteine levels may be a risk marker for placental vascular disease and maternal preeclampsia.

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