The Effects of Metformin, Ethinyl Estradiol/Cyproterone Acetate, and Metformin Ethinyl Estradiol/Cyproterone Acetate Combination Therapy on Carotid Artery Intima-media Thickness in Patients with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Olgularda Metformin, Etinil Estradiol/Siproteron Asetat ve Metformin-etinil Estradiol/Siproteron Asetat Kombinasyonu Tedavisinin Karotis Arter İntima Media Kalınlığı Üzerine Etkileri

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ABSTRACT

Introduction: Patients with polycystic ovary syndrome (PCOS) are in the risk group for early-onset cardiovascular disease. There are few studies evaluating physiological and inflammatory cardiovascular risk factors in PCOS. Our study aimed to measure carotid intima-media thickness (IMT) in PCOS cases and to assess the effects of metformin, ethinyl estradiol/cyproterone acetate (EE/CA) and metformin + EE/CA combination therapy on carotid IMT, insulin resistance, C-reactive protein (CRP), apelin and adiponectin.

Methods: Basal carotid IMT, insulin resistance [Homeostasis model assessment insulin resistance (HOMA-IR)], apelin, adiponectin, and CRP values were evaluated in 60 women with PCOS and 43 healthy volunteers between the ages of 18 and 45. After baseline evaluation, patients were divided into metformin (n=20), EE/CA (n=20) and metformin + EE/CA (n=20) treatment groups. Treatment regimens were administered for six months. At the end of the treatment, the same parameters were re-evaluated.

Results: Compared with the control group, CRP (p=0.003), HOMA-IR (p=0.004) and IMT (p=0.049) were significantly higher, and adiponectin (p=0.002) and apelin (p=0.031) levels were significantly lower in patients with PCOS. At the end of the six-month treatment, the adiponectin level in the metformin (p=0.012) and metformin + EE/CA groups (p=0.012), and the apelin level in the metformin (p=0.024), EE/CA (p=0.024) and metformin + EE/CA groups (p=0.024) were significantly higher. There was no statistically significant change in CRP level in all

ÖZ

Amaç: Polikistik over sendromu (PKOS) erken dönemde ortaya çıkan kardiyovasküler hastalık için risk grubundadır. PKOS'de, fizyolojik ve enflamatuvar kardiyovasküler risk faktörlerini değerlendiren az sayıda çalışma vardır. Çalışmamızın amacı, PKOS olgularında karotis intima media kalınlığını (İMK) ve ayrıca metformin, etinil estradiol/siproteron asetat (EE/SA) ve metformin + EE/SA kombinasyon tedavisinin karotis İMK, insülin direnci, C-reaktif protein (CRP), apelin ve adiponektin üzerine etkilerini değerlendirmektir.

Yöntemler: Çalışmamıza katılan 18-45 yaş arasındaki 60 PKOS'li kadında ve 43 sağlıklı gönüllü kadında bazal karotis İMK, insülin direnci [Homeostasis model assessment insulin resistance (HOMA-IR)], apelin, adiponektin ve CRP değerlendirmeleri yapıldı. Bazal değerlendirmeden sonra, hastalar metformin (n=20), EE/SA (n=20) ve metformin + EE/SA (n=20) tedavi gruplarına ayrıldı. Tedavi rejimleri 6 ay boyunca uygulandı. Tedavi sonunda aynı parametreler tekrar değerlendirildi.

Bulgular: Kontrol grubuyla karşılaştırıldığında PKOS'li olguların CRP (p=0,003), HOMA-IR (p=0,004), İMK (p=0,049) değerleri istatistiksel olarak anlamlı derecede daha yüksek, adiponektin (p=0,002), apelin (p=0,031) düzeyleri kontrol grubundan istatistiksel olarak daha düşük saptandı. Altı aylık tedavinin sonunda adiponektin seviyesi metformin (p=0,012) ve metformin + EE/SA grubunda (p=0,012), apelin seviyesi ise metformin grubu (p=0,024), EE/SA grubu (p=0,024), ve metformin + EE/SA grubunda (p=0,024) anlamlı olarak yüksek saptandı. Üç tedavi grubunda da CRP seviyesinde istatistiksel olarak anlamlı değişim yoktu (p>0,05). Üç tedavi grubunda

treatment groups (p>0.05). There was no statistically significant change in carotid IMT value in all treatment groups (p>0.05).

Conclusion: According to these results, we can say that women with PCOS have subclinical atherosclerosis and that metformin treatment has a positive effect on subclinical atherosclerosis.

Keywords: Polycystic ovary syndrome, carotid artery intima-media thickness, insulin resistance, apelin, adiponectin, C-reactive protein

da karotis İMK değerinde istatistiksel olarak anlamlı değişim yoktu (p>0,05).

Sonuç: Bu sonuçlara göre PKOS'de subklinik aterosklerozun varlığını ve metformin tedavisinin subklinik ateroskleroz üzerine olumlu etki gösterdiğini söyleyebiliriz.

Anahtar Kelimeler: Polikistik over sendromu, karotis arter intima media kalınlığı, insülin direnci, apelin, adiponektin, C-reaktif protein

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder (5-10%) in women of reproductive age (1). It was first described by Stein and Leventhal (2) in 1935. The diagnosis of the disease, the symptoms of which spread over a broad spectrum, was based on clinical signs and symptoms and biochemical markers, and today there is no consensus on diagnostic criteria (3).

First, in 1990, the National Institutes of Health Consensus Conference specified the diagnostic criteria for PCOS as the exclusion of other causes of hyperandrogenism, characterized by menstrual irregularity, increased androgen level, and adrenal hyperplasia. Insulin resistance, increased luteinizing hormone/follicle-stimulating hormone ratio, and ultrasonographic symptoms have been identified as possible criteria (4).

In 2003, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine convened in Rotterdam, Netherlands, to rearrange the diagnostic criteria for the diagnosis of PCOS. Since PCOS is a syndrome, it was decided after this meeting that a single criterion was not sufficient for clinical diagnosis and that at least two of the following criteria should be present after the exclusion of androgen excess and other medical causes causing menstrual irregularity:

1. Oligo/anovulation,

2. Clinical or biochemical hyperandrogenemia findings,

3. PCOS appearance in at least one ovary on ultrasound (US) (defined as more than 12 2-9 mm, follicles in the ovary or ovarian volume more than 10 mL) and exclusion of other diseases (5).

In 2009, the diagnostic criteria of PCOS were reorganized by the Androgen Excess Society.

Androgen Excess Society Criteria

1. Hyperandrogenism: Hirsutism and/or hyperandrogenemia,

2. Ovarian dysfunction: Oligo-anovulation and/or polycystic ovaries,

3. Exclusion of other hyperandrogenemia-causing conditions (such as late-onset congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, thyroid dysfunction, and hyperprolactinemia) (6).

PCOS progresses with clinical symptoms such as dysfunctional bleeding, infertility, hirsutism, and acne. The most common of these is menstrual irregularity due to anovulation (7). Also, there are women with regular menstrual cycles despite high androgen levels (8). In PCOS,

hyperandrogenism is evaluated with clinical (hirsutism, acne, androgenic alopecia) and biochemical (increase in serum total and free testosterone levels) findings (6). There is an 80% PCOS with hyperandrogenemia. Ovaries are enlarged (volume >10 mL), and polycystic ovaries (12 or more follicles with a size of 2-9 mm), can be detected. It is sufficient to see them in a single ovary (9).

Many patients with PCOS have insulin resistance independent of body mass index. The risk of developing type 2 diabetes mellitus (DM) in patients with PCOS has been found to increase 2-5 times (10). Also, the frequency of metabolic syndrome is higher in patients with PCOS than in the normal population. The risk of cardiovascular disease (CVD) increases in patients with DM and metabolic syndrome (11).

Adiponectin is a newly defined adipocytokine expressed in adipocytes. Adiponectin is effective in insulin resistance and in preventing the development of type 2 DM. It plays a vital role in vascular endothelial dysfunction and the pathogenesis of atherosclerosis. In clinical studies, adiponectin has been shown to have a protective effect from atherosclerosis. Serum adiponectin level is low in women with type 2 DM, obesity, and insulin resistance. In studies with PCOS, plasma adiponectin level was also found to be low (12).

Apelin has been shown to have an expression in the central nervous system, lung, adipose tissue, heart, and breast. Apelin is thought to play a role in the regulation of insulin and glucose levels (13).

Many studies have revealed that those with increased C-reactive protein (CRP) levels have a much higher risk of CVD, including stroke, sudden death, and peripheral vascular disease (14,15). The majority of the studies in PCOS have been shown to increase the level of CRP (15).

Carotid intima-media thickness (IMT) measurement is one of the methods that detect the atherosclerotic process in the asymptomatic period and is also a technique showing endothelial dysfunction and early atherosclerotic changes. Studies investigating the subclinical CVD in PCOS by detecting the presence of coronary artery calcification and carotid IMT were performed.

A significant correlation was observed between carotid IMT and testosterone, and androgens were thought to play an essential role in the development of atherosclerosis. It is predicted that atherogenic potential is increased in patients with PCOS in relation to androgen excess, regardless of obesity (16).

Treatment goals in PCOS can be listed as correction of hyperandrogenism, menstrual dysfunction, and providing fertility. Oral contraceptive agents

(OCS), long-acting GnRH analogs, and insulin sensitivity-enhancing agents can be used in androgen-reducing therapy. After understanding that insulin resistance has a significant effect on the development of PCOS in recent years, agents that increase insulin sensitivity have taken their place in the treatment options (17). Insulin sensitivity increasing drugs such as metformin and glitazones have started to be used intensively in the treatment of PCOS (17).

In this study, we aimed to determine whether the carotid IMT, homeostatic model assessment of insulin resistance (HOMA-IR), apelin, adiponectin and CRP levels in PCOS cases differ from the control group, and to evaluate how these parameters respond to three different treatment approaches, including Metformin, Ethinyl estradiol/cyproterone acetate (EE/CA) and metformin + EE/CA.

Methods

Ethics Committee Approval and Project Support

Written approval was obtained for the study from Kırıkkale University Faculty of Medicine Local Ethics Committee on 03.06.2009 with the number of 2009/089.

Selection of the Study Cohort

A total of 60 patients diagnosed with PCOS in accordance with the criteria of Rotterdam 2003, aged between 18 and 45, who applied to the Kırıkkale University Faculty of Medicine Department of Endocrinology and Metabolic Diseases, and 43 age-matched, healthy volunteers were included in our study (5). The patients were randomized to the treatment group according to their order of admission.

Twenty patients received 2x1 g/day metformin, 20 patients received 0.035 mg EE / 2 mg CA / d 1x1 (between 5th-25th day of menstruation, 21 days) and 20 patients received 2x1 g/g metformin + EE/CA (between 5th-25th day of menstruation, 21 days) combined therapy. Baseline examinations were repeated at the 6th month of treatment. These procedures were performed only once in the control group.

Before starting the study, all patients in the PCOS and control groups were informed about the study, and patient consent was obtained. Patients with coronary artery disease, congestive heart failure, late-onset congenital adrenal hyperplasia, Cushing's syndrome, hypothyroidism, hyperthyroidism, type 2 DM, androgen-secreting ovarian or adrenal tumors, and patients receiving antiandrogen treatment and treatment affecting insulin sensitivity were also not included in the study.

Anthropometric Measurements, Collection of Samples, Laboratory Analysis Methods and Vascular Evaluations

In all cases included in the study, fasting blood glucose and fasting serum insulin levels were measured after at least 10 hours of fasting. HOMA-IR was calculated using the formula fasting blood glucose (mmol/L) x fasting insulin (µU/L)/22.5 (18).

CRP was evaluated with the turbidometric method. Serum adiponectin (Human Adiponectin AssayPro ELISA; Biotech Inst Spect) and apelin (Human Apelin Biotech in ELx Autostrip Washer) levels were measured collectively.

The menstrual statuses of the patients were evaluated according to the 6 month menstrual cycles before the study. Menstrual patterns were defined as "regular" (21-35 days), "irregular" (35-180 days), "polymenorrhea" (21 days or less), "oligomenorrhea" (35-180 days) and "amenorrhea" (longer than 180 days) (3). Pelvic US evaluations of all cases were performed on the 3rd-5th days of the menstrual cycle.

For the carotid artery IMT measurement, patients were positioned in the supine position with their heads tilted backward. Measurements were made in the right and left carotid arteries by determining a 1 cm segment within the first 2 cm distal region from the common carotid artery bulb using the General Electric Vivid S5 ultrasonography device and 12L Doppler ultrasonography probe. Based on the far-edge measurement method of the US device, the maximum and mean carotid IMT values of the segment under consideration were determined. The measurement was performed for both common carotid arteries.

Statistical Analysis

All data obtained from the study were analyzed using SPSS 17.0. All data were expressed as mean ± standard deviation (SD). After conducting descriptive statistical analyses (frequency, percentage distribution, mean ± SD), the suitability of variables to normal distribution was evaluated with the Kolmogorov-Smirnov test. Initially, the difference between treatment groups was tested with one-way ANOVA. The levels of biochemical, inflammatory, and carbohydrate metabolism parameters of the groups before and after the treatment were analyzed with the Wilcoxon signed-rank test. $P<0.05$ values were considered statistically significant.

Results

Sixty PCOS patients and 43 volunteers were included in the study. There were 20 patients receiving metformin treatment, 20 patients receiving EE/CA treatment, and 20 patients receiving metformin + EE/CA combination therapy in the PCOS treatment group. Among patients receiving metformin treatment, treatment was discontinued due to B12 deficiency in two patients and severe gastrointestinal complaints in one patient. In one patient receiving EE/CA treatment, treatment was discontinued due to elevated liver enzymes. Also, four patients from the Metformin group, nine patients from the EE/CA group and five patients from the metformin + EE/CA combination group were excluded from the study because they did not come to the 6th month controls and they were not included in the statistical evaluation. The total number of patients who came for control in the 6th month of treatment was 38 (63.3%). The number of patients who completed the study was 13 in the metformin group, 10 in the EE/CA group, and 15 in the metformin + EE/CA combination group.

Comparison of the Pre-treatment Parameters of Individuals in the PCOS and Control Groups

Compared with the control group, CRP (p=0.003), HOMA-IR (p=0.004) and carotid IMT (p=0.049) values of PCOS cases were found to be significantly higher. Adiponectin (p=0.002) and apelin (p=0.031) levels of PCOS cases were significantly lower than the control group (Table 1).

Comparison of Pre-treatment Parameters of Three Treatment Groups

A statistically significant difference was found between the levels of apelin (p=0.040), adiponectin (p=0.007) and CRP (p=0.019) in the three treatment groups. There was no statistically significant difference between the carotid IMT and HOMA-IR levels of the three groups (p>0.05) (Table 2).

Comparison of Parameters of Three Treatment Groups Measured at Six Months of Treatment

There was a statistically significant difference between IMT (p=0.018), adiponectin (p=0.010) and CRP (p=0.019) levels at the 6th month of treatment of the three treatment groups (Table 3). The significant difference between IMT values was found to be due to the mean value of the metformin group being higher than the mean value of the EE/CA group (p=0.046, respectively). It was found that the significant difference between adiponectin levels was due to the mean level of the metformin group being lower than the mean level of the EE/CA group (p=0.033). It was found that the significant difference between CRP levels was due to the mean level of the metformin group being higher than the mean level of the metformin + EE/CA group (p=0.002). There was no statistically significant difference between HOMA-IR and apelin levels of the three groups (p>0.05).

Comparison of the Parameters Before and After Treatment in Treatment Groups

Metformin treatment group: Significant decrease in HOMA-IR (p=0.048) values and significant increase in apelin (p=0.024) and adiponectin

(p=0.012) levels were detected at the end of the 6th month of treatment. There was no significant difference between IMT values and CRP levels before and after treatment (p>0.05) (Table 4).

EE/CA treatment group: There was a significant increase in apelin levels (p=0.024) after six months of treatment. There was no significant difference between the carotid IMT, HOMA-IR, adiponectin, and CRP levels of these cases before and after treatment (p>0.05) (Table 5).

Metformin + EE/CA treatment group: There was a significant increase in apelin (p=0.024) and adiponectin (p=0.012) levels after 6 months of treatment. There was no significant difference between IMT, HOMA-IR, and CRP levels before and after treatment (p>0.05) (Table 6).

Discussion

In patients with PCOS, carotid IMT has been evaluated in many studies. Talari et al. (19) found that the carotid artery IMT increased in patients with PCOS compared to the control group). In the study conducted by Talbott et al. (20), the increase in carotid IMT was shown among women over 45 years of age with PCOS compared to the control group, but not in women with PCOS in the 30-44 years age group. Similar to these studies, we found in our study that the carotid IMT value was higher in PCOS cases than the control group.

Table 1. Comparison of laboratory parameters of individuals in PCOS and control groups

	PCOS (n=60)	Control (n=43)	p
Adiponectin (µg/mL)	10.64±6.76	14.61±5.80	0.002
Apelin (ng/mL)	2.63±2.89	3.78±2.20	0.031
Carotid IMT (mm)	0.42±0.078	0.39±0.06	0.049
CRP (mg/dL)	91.35±27.05	81.79±28.27	0.003
HOMA-IR	3.01±1.78	2.15±0.903	0.004

PCOS: polycystic ovary syndrome, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance

Table 2. Comparison of pre-treatment values of laboratory parameters of the treatment group

Groups				
	Metformin (n=13)	EE/CA (n=10)	Metformin + EE/CA (n=15)	p
	Mean ± SD	Mean ± SD	Mean ± SD	
Carotid IMT (% change)	0.45±0.08	0.40±0.09	0.44±0.05	NS
HOMA-IR	3.60±1.89	2.65±2.00	2.91±1.55	NS
Apelin (ng/mL)	2.69±4.03	3.45±3.49	1.81±0.47	0.040
Adiponectin (µg/mL)	6.37±2.63	12.99±7.67	9.00±5.97	0.007
CRP (mg/dL)	8.94±9.21	2.90±3.62	1.87±1.39	0.019

IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, SD: standard deviation, NS: not significant

Table 3. Comparison of parameters of three treatment groups measured at six months of treatment

Groups				
	Metformin (n=13)	EE/CA (n=10)	Metformin + EE/CA (n=15)	p
	Mean ± SD	Mean ± SD	Mean ± SD	
IMT	0.45±0.06*	0.39±0.05	0.40±0.05	0.018
HOMA-IR	2.80±1.47	2.63±2.58	2.51±1.66	AD
Apelin (ng/mL)	3.59±2.24	4.71±3.81	4.18±1.88	AD
Adiponectin (µg/mL)	9.53±2.93*	14.91±7.73	13.92±8.86	0.010
CRP (mg/dL)	6.73±6.92**	3.30±2.83	2.43±1.92	0.019

*Significant difference between metformin and EE/CA groups (p<005)
**Significant difference between metformin and metformin + EE/CA groups (p<005).
IMT: intima media thickness, SD: standard deviation, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate

Table 4. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving metformin treatment

Metformin

	Pre-treatment (n=13)	Post-treatment (n=13)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.45±0.08	0.45±0.06	NS
HOMA-IR	3.60±1.89	2.80±1.47	0.048
Apelin (ng/mL)	2.69±4.03	3.59±2.24	0.024
Adiponectin (µg/mL)	6.37±2.63	9.53±2.93	0.012
CRP (mg/dL)	8.94±9.21	6.73±6.92	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, NS: not significant

In their study, Song et al. (21) found that PCOS cases had high insulin resistance. In our study, we found that HOMA-IR values in PCOS cases were higher than the control group.

The presence of a correlation between serum apelin levels and insulin resistance in PCOS is still controversial (22). Chang et al. (23) evaluated both obese and non-obese cases in their study and found that the level of apelin in PCOS patients was lower than the control group. We found lower levels of apelin in PCOS cases than the control group. To date, no study investigating the effect of metformin and EE/CA treatments on apelin in patients with PCOS has been conducted. On the other hand, there was a significant increase in serum apelin levels in all treatment groups (metformin, EE/CA, and metformin + EE/CA) in PCOS at the end of the 6th month of treatment. We think that this result is vital since it is the first and significant result related to the subject in the literature.

Serum adiponectin levels in PCOS patients have been investigated in many studies. In the study conducted by Demirci et al. (24), plasma adiponectin level was found to be low in patients with PCOS. In our study, adiponectin level (p=0.002) of patients with PCOS was found statistically lower than the control group.

The majority of the studies in PCOS have shown increased levels of CRP (19,25). In a meta-analysis, it was found that increased CRP levels decreased in patients with PCOS after metformin treatment (25). In our study, we found that CRP level was significantly higher in patients with PCOS compared to the control group.

In a study by Sahin et al. (26), 20 women with PCOS were compared with 20 age-matched healthy women, and it was shown that six months of metformin 2550 mg/day treatment did not cause a significant change in the carotid IMT value in women with PCOS. Similar to the study of Sahin et al. (26), we determined that six months of metformin 2000 mg/day treatment did not cause a significant change in the carotid IMT value. However, we found that six months of metformin 2000 mg/day treatment decreased the HOMA-IR value and increased the levels of adiponectin and apelin.

A combination of OCS and metformin is a crucial treatment option in obese and non-obese PCOS patients. In a study conducted by Mitkov et al. (27), it was shown that combined EE/CA treatment with metformin did not impair insulin sensitivity. In our study, we found that six months of treatment with metformin and EE/CA did not change the HOMA-IR value. We found that while PCOS cases treated with metformin + EE/CA did not change carotid IMT, HOMA-IR and CRP levels, apelin, and adiponectin levels increased significantly.

Conclusion

We detected increased carotid IMT in PCOS cases, which is an early symptom of atherosclerosis. However, in patients with PCOS, serum levels of adiponectin and apelin were low, and CRP levels were high. We did not find a significant decrease in carotid IMT in all three treatment groups after treatment. We found an increase in serum apelin levels in all three treatment groups, and we found that this is the first and significant result in the literature. While adiponectin level increased in metformin and metformin + EE/CA group, it did not change in the EE/CA group. According to these results, we think that new, large-scale, prospective, and randomized studies are needed to evaluate subclinical and clinical atherosclerosis in PCOS cases.

Table 5. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving ethinyl estradiol/cyproterone acetate treatment

EE/CA

	Pre-treatment (n=10)	Post-treatment (n=10)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.40±0.09	0.39±0.05	NS
HOMA-IR	2.65±2.00	3.63±3.58	NS
Apelin (ng/mL)	3.45±3.49	4.72±3.81	0.024
Adiponectin (µg/mL)	12.99±7.67	14.91±7.73	NS
CRP (mg/dL)	2.90±3.62	3.30±2.83	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, NS: not significant

Table 6. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving metformin + EE/CA treatment

Metformin + EE/CA

	Pre-treatment (n=15)	Post-treatment (n=15)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.44±0.05	0.40±0.05	NS
HOMA-IR	2.91±1.55	2.51±1.66	NS
Apelin (ng/mL)	1.81±0.47	4.18±1.88	0.024
Adiponectin (µg/mL)	9.00±5.97	13.91±8.86	0.012
CRP (mg/dL)	1.87±1.39	2.43±1.92	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, NS: not significant

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