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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Low plasminogen activator inhibitor-1 levels in thyroid carcinoma: uPA/PAI-1 paradox in cancer proggression

Tiroid kanserinde düşük plazminojen aktivatör inhibitör-1 düzeyleri: Kanser gelişiminde uPA/PAI-1 paradoksu

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ÖZ

Amaç: Plazminojen aktivatör inhibitor-1 (PAI-1) hücre migrasyon ve apoptozisinin güçlü inhibitörüdür. Çoğu kanser türünde yüksek PAI-1 düzeyleri tespit edilmiştir. Bir çok çalışmada tiroid kanserlerinde artmış PAI-1 ekspresyonu ve bunun kötü klinik sonuçlarla ilişkili olduğu gösterilmiştir. Bu çalışmada papiller tiroid kanserlerinde(PTK) serum PAI-1 düzeylerini ve bunun PTK gelişimi ve boyutu üzerine etkisini araştırmak amaçlanmıştır.

Materyal ve Metod: Elli dört papiller tiroid kanser hastası (7 erkek, 47 kadın) ve 24 sağlıklı kontrol (6 erkek, 18 kadın) çalışmaya dahil edilmiştir. Gruplar dermografik, antropometrik ve biyokimyasal veriler ile serum PAI-1 düzeyleri açısından karşılaştırılmıştır. Serum PAI-1 düzeyleri enzim-bağlı immünabsorban kit (ELİZA) ile çalışılmıştır.

Results: Ortalama yaş PTK ve kontrol grubunda benzerdi (42.4 ± 10.1 ile 42.5 ± 8.9, p:0.794). Serum PAI-1 düzeyleri sağlıklı kontrollerle karşılaştırıldığında PTK hastalarında düşük olarak saptandı (241.34 ± 107.82 ile 327.24 ± 138.51, p:0.011). Vücut kitle indeksi (VKİ), Homa-IR, TSH, sT4, anti-TPO, anti-Tg, total kolesterol, trigliserid, HDL-kolesterol, LDL-kolesterol, kalsiyum, fosfor, 25-OH-VitD, parathormon, glukoz, insülin düzeyleri gruplar arasında benzer olarak bulundu (p>0.05). PTK hastalarında PAI-1 düzeyleri ile parathormon(PTH) dışında klinik, biyokimyasal ve hormonal parametreler arasında korelasyon bulunamadı (r:-0.446, p:0.0027).

Conclusions: Papiller tiroid kanserli hastalarda serum PAI-1 düzeyleri düşük olarak bulunmuştur. Bizim sonuçlarımız ürokinaz plazminojen aktivatör aktivitesini inhibisyon kapasitesinden dolayı kanser ilerlemesini baskılaması beklentisi tezini destekleyebilir.

Anahtar Kelimeler: Plazminojen aktivatör inhibitor-1, papiller tiroid kanseri, ürokinaz plazminojen aktivatörü

ABSTRACT

Aim: Plasminogen activator inhibitor-1 (PAI-1) potently inhibits cell migration and apoptosis. Increased levels of PAI-1 were demonstrated in a great variety of cancers. Many studies demonstrated increased expression of PAI-1 in thyroid cancer and its relation to unfavorable clinical outcome. In this study, we aimed to investigate serum PAI-1 levels in patients with papillary thyroid carcinoma (PTC) as well as its effect in development and size of PTC.

Material and methods: Fifty-four papillary thyroid cancer patients (7 male, 47 female) and 24 healthy controls (6 male, 18 female) were enrolled in the study. Groups were compared by demographic, anthropometric, biochemical data, and by serum ghrelin levels. Serum PAI-1 levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: Mean age were similar between PTC and control group (42.4 ± 10.1 to 42.5 ± 8.9, p:0.794). Serum PAI-1 levels were lower in patients with PTC when compared to healthy controls (241.34 ± 107.82 to 327.24 ± 138.51, p:0.011). BMI, Homa-IR and TSH, FT4, anti-TPO, anti-Tg, total cholesterol, triglyceride, HDL-Cholesterol, LDL-Cholesterol, calcium, phosphorus, 25-OH-VitD, parathormone, glucose, insülin concentrations were similar between groups (p>0.05). PAI-1 concentrations were not correlated with clinical, biochemical and hormonal parameters except parathormone (PTH) concentrations in PTC group (r:-0.446, p:0.0027).

Conclusions: Serum PAI-1 levels were lower in patients with papillary thyroid carcinoma. Our results might support the thesis of PAI-1 is expected to suppress cancer progression due to its ability to inhibit urokinase plasminogen activator activity.

Keywords: Plasminogen activator inhibitor-1, papillary thyroid carcinoma, urokinase plasminogen activator

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Introduction

The main urokinase plasminogen activating system (uPAS) be composed of the urokinase plasminogen activator (uPA), its cognate receptor (uPAR), and two main plasminogen activator inhibitors, PAI-1 and PAI-2. uPA converts plasminogen into the serine protease plasmin that play role in several pathophysiological processes including angiogenesis, tumor progression and dissemination (1). PAI-1 potently inhibits cell migration (2,3) and apoptosis (4). However, tumor growth and vascularization were discovered to decrease in PAI-1 knockout mice (5) and increased levels of PAI-1 were demonstrated to predict poorer rather than better clinical outcome in a great variety of cancers including gastric (6), colorectal (7), breast (8), ovarian (9), and lung cancer (10). In contrary to these findings, Chen et al observed that both tumor growth and angiogenesis were inhibited in PAI-1 overexpressed prostate carcinoma cells (11). Eitzman DT et al demonstrated that growth of murine melanoma tumors' metastasis was unaffected by the presence of PAI-1 expression (12). Almholt K et al demonstrated PAI-1 status did not influence tumor growth and vascularization in transgenic mice (13). Adenovirus-mediated PAI-1 gene expression was discovered to decrease the tumor growth, migration and metastasis in vivo (14–16). Thyroid carcinoma cell lines and papillary thyroid carcinoma (PTC) tissues overexpress PAI-1 both mRNA and protein levels (17). Ito et al demonstrated that most thyroid carcinomas diffusely express plasminogen activator inhibitor type 1 (PAI-1) however no association with prognosis was observed (18). PAI-I evaluated by ELISA were demonstrated to be higher in the cytosolic fraction of malignant thyroid tumor tissues when compared to benign thyroid tumor tissues (19). PAI-1 evaluated by ELISA was shown to be higher in paired cytosol samples of malignant thyroid tumors and associated with worse prognosis (20). However, to our knowledge no studies evaluated serum PAI-1 levels in thyroid cancer. In this study, we aimed to investigate serum plasminogen activator inhibitor-1 (PAI-1) levels in patients with papillary thyroid carcinoma (PTC) as well as its effect in development and size of PTC.

Material and Methods

Study population

54 patients (7 male, 47 female) with papillary thyroid cancer (PTC) and 24 age, sex, and BMI-matched controls (6 male, 18 female) were included in the study. Ethics committee approval and written informed consent of participants were obtained prior to the study. The diagnosis of PTC was confirmed by histopathologic documents. Blood was collected from patients before surgery and from healthy individuals as the normal controls. Subjects with other cancers and autoimmune disorder, hypertension, hepatic or renal dysfunction, diabetes mellitus, or any other inflammatory or medical condition were excluded.

Clinical, biochemical and hormonal measurements

Weight, height, waist circumference (WC), hip circumference (HC) and systolic and diastolic blood pressure (BP) were measured. WC was determined by measuring the narrowest point between the costal margin and iliac crest at the end of a normal expiration. The BMI was calculated as weight (kg)/height $(m)^2$. After an 8-12 hour overnight fast, the venipuncture was performed between 8:00 am and 9:00 am and blood samples were collected into plain tubes. Blood samples were centrifuged at 2.500 g for 15 min within 30 min of collection, and serum samples were stored at –80 °C until analysis. Serum levels of glucose, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), creatinine, thyroid stimulating hormone (TSH), free T4 (fT4) were also measured.

Measurement of plasminogen activator inhibitor-1

Measurements of PAI1 (eBioscience, USA) were performed in an ELISA reader EPOCH system (BioTek Instruments, Inc, USA) using the commercially available ELISA kit in accordance with the manufacturers' instructions.

Statistical Analysis

The descriptive values for the data obtained are expressed in mean ± SD, numbers, and percentage frequencies. Kolmogorov-Smirnov test was used to review whether the numerical measurements exhibit a normal distribution and it has been determined that the numerical variables are distributed normally. Student's t-test and Mann-Whitney U-test were used to compare differences between two independent groups with normal and non-normal distributions, respectively. The relationships between individual numerical properties were reviewed by Pearson correlation analysis in the patient and control groups. $p \leq 0.05$ was used as the statistical significance level and IBM SPSS 20.0 was processed for the calculations.

Results

Mean age was similar between PTC and control group (42.4 ± 10.1 to 42.5 ± 8.9, p:0.794). Serum PAI-1 levels were lower in patients with PTC when compared to healthy controls (241.34 ± 107.82 to 327.24 ± 138.51, p:0.011) (Figure 1). BMI, Homa-IR and TSH, FT4, anti-TPO, anti-Tg, total cholesterol, triglyceride, HDL-Cholesterol, LDL-Cholesterol, calcium, phosphorus, 25-OH-VitD, parathormone, glucose, insülin concentrations were similar between groups (p>0.05). Mean HsCRP concentration was higher in PTC group when compared with control group (p: 0.021). Waist circumference was higher in PTC group when compared to control group (94.10 ± 12.34 to 86.31 ± 9.63, p:0.0094) (Table 1). PAI-1 concentrations were not correlated with BMI, TSH, FT4, anti-TPO, anti-Tg, total cholesterol, triglyceride, HDL-Cholesterol, LDL-Cholesterol, calcium, phosphorus, 25-OH-VitD, glucose, insülin, Homa-IR in PTC group (p>0.05). Parathormone concentrations were negatively correlated with PAI-1 concentrations (r:-0.446, p:0.0027) (Table 2).

Table 1.

	PTC Group		Control Group		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Age (years)	42.37	10.15	41.75	9.41	0.794
Height (cm)	161.13	7.05	162.83	8.50	0.654
Weight (kg)	75.57	15.00	76.80	11.10	0.829
BMI (kg/m^2)	29.14	5.69	28.14	2.80	0.526
Waist circumference (cm)	94.10	12.34	86.31	9.63	**0.0094**
TSH (µIU/mL)	2.31	4.20	1.84	0.99	0.457
FT4 (ng/dL)	1.17	0.30	1.08	0.16	0.086
Anti-TPO (IU/mL)	104.25	260.24	139.39	324.41	0.695
Anti-Tg (U/mL)	44.97	81.96	46.80	72.06	0.934
Total cholesterol (mg/dL)	207.62	41.85	191.17	39.21	0.141
Triglyceride (mg/dL)	145.55	77.68	128.71	80.46	0.418
HDL-Cholesterol (mg/dL)	52.58	11.74	48.41	10.19	0.130
LDL-Cholesterol (mg/dL)	120.72	39.01	117.00	33.20	0.673
Calcium (mg/dL)	9.36	0.58	9.58	0.46	0.100
Phosphorus (mg/dL)	3.60	0.86	3.38	0.33	0.113
25-OH-VitD (ng/mL)	14.29	7.53	15.95	5.53	0.307
Parathormon (pg/ml)	44.86	20.86	50.88	28.53	0.436
Glucose (mg/dL)	87.26	24.57	85.79	10.07	0.710
Insulin (mIU/L)	11.32	5.25	13.15	14.33	0.611
Homa-IR	2.53	1.25	2.98	3.99	0.652
HsCRP (mg/l)	4.36	4.91	1.92	2.78	**0.021**
PAI-1 (ng/mL)	241.34	107.82	327.24	138.51	**0.011**

Discussion

PAI-1 is the main physiological inhibitor of plasminogen activation by uPA and tPA. uPA is considered to initiate releasing of proteolytic enzymes in tumors, thus facilitates cancer-cell

invasion into the surrounding normal tissue via degradation of basement membranes and extracellular matrix (21). uPA is causally involved in promoting cancer invasion and metastasis (22).

Table 2. The correlation between PAI-1 concentrations and clinical, biochemical and hormonal parameters in PTC group

Variable	Correlation coeficient	p-value
Age (years)	-0.2201	0.1169
BMI (kg/m^2)	0.0954	0.5331
TSH (µIU/mL)	0.0341	0.8163
FT4 (ng/dL)	-0.0429	0.7698
Anti-TPO (IU/mL)	0.1796	0.2675
Anti-Tg (U/mL)	0.2436	0.0883
Total cholesterol (mg/dL)	-0.0372	0.7958
Triglyceride (mg/dL)	0.0827	0.5642
HDL-Cholesterol (mg/dL)	-0.0421	0.7693
LDL-Cholesterol (mg/dL)	-0.0619	0.6659
Calcium (mg/dL)	-0.2456	0.0793
Phosphorus (mg/dL)	0.2584	0.0672
25-OH-VitaminD (ng/mL)	0.0474	0.7387
Parathormon (pg/ml)	-0.4457	**0.0027**
Glucose (mg/dL)	-0.0906	0.5232
Insulin (mIU/L)	0.2623	0.0658
Homa-IR	0.0861	0.5519
Homocysteine	0.1546	0.4063
HsCRP(mg/l)	0.1127	0.4828
Waist circumference	-0.0414	0.7896

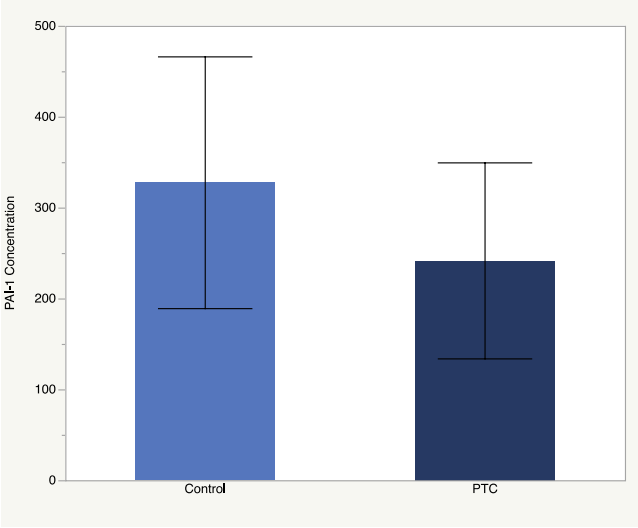

Figure 1: Plasma levels of PAI-1 in study groups

PAI-1 is expected to suppress cancer progression due to its ability to inhibit uPA activity however, PAI-1 were found to independent prognostic factors. There are conflicting results about the effect of PAI-1 on tumor growth and metastasis. High PAI-1 levels predict adverse outcome in multiple cancer types including breast, ovary, cervix, endometrium, stomach, colon, lung, bladder, kidney, brain, and soft-tissue (23,24). Regulation of plasmin-mediated proteolysis (25,26) and/or cell migration (27) by PAI-1 is considered to be the reason of PAI-1 induced angiogenesis. In contrary to these findings, PAI-1 overexpression in prostate carcinoma cells inhibits both tumor growth and angiogenesis (11). Moreover, some studies demonstrated that PAI-1 status did not effect tumor (12,13). Administration of pharmacological level of PAI-1 was demonstrated to prevent the angiogenesis, tumor growth, and dissemination (28,29). Stefansson et al demonstrated that exogenously added PAI-1 at therapeutic concentrations was a potent inhibitor of angiogenesis (30) In vivo studies demonstrated that adenovirus-mediated PAI-1 gene expression decreased the tumor growth, migration, and metastasis (14–16).

Ulisse S et al compared the expression analysis of uPA, uPAR and PAI-1 in human PTC specimens compared to those of normal matched tissues using quantitative RT-PCR and Western blot. They found that higher expression of uPA, uPAR, and PAI-1, at both mRNA and protein levels, correspond to malignant transformation of the human thyrocyte (17). The highest cytosolic level of PAI-1 was observed in samples with thyroid cancer compared to benign thyroid diseases (19). These findings were supported by Horvatic Herceg G et al that reported cytosolic PAI-1 levels were significantly higher in differentiated thyroid tumors compared to normal tissues and patients with higher PAI-1 have a poorer prognosis (31). Ulisse S et reported that the uPA, uPAR, and PAI-1 mRNA levels were significantly higher in patients with papillary, medullary, follicular and anaplastic thyroid cancer compared with normal matched tissues (32). Another study reported most thyroid carcinomas diffusely express PAI-1, but no relation with

clinicopathological parameters was observed (18). Altogether, these studies demonstrated increased expression of uPAS components in thyroid cancer and the degree of overexpression positively correlates with prognostic factors including tumor size, lymph node, or distant metastases (1). It was demonstrated that inhibition of uPA and uPAR reduces proliferation, migration and invasive capacity of thyroid cancer cells (33). In contrary to these results, we found that PAI-1 concentrations were lower in our patient group.

PAI-1 is an inhibitor of uPA and both uPA and PAI-1 appears to be markers of tumor aggressiveness. This apparent paradox could be due to the multiple and complex pathways in which uPA, uPAR and particularly PAI-1 affects tumor biology (34,35). Although inhibition of uPA, uPAR or PAI-1 by several approaches has demonstrated to decrease the growth and metastasis of experimental tumors in animals, this inhibition has not been able to show any effect on human cancers (36). In light of this information, we still don't know for sure how PAI-1 affect tumor behavior in cancer which might partly explain our on the contrary results in this particular study.

In conclusion, serum PAI-1 levels are demonstrated to be significantly lower in thyroid cancer patients compared to healthy controls. Our findings were not consistent with the results of other studies that evaluate the effect of PAI-1 in thyroid cancer. However, our results might support the concept of PAI-1 is expected to suppress cancer progression due to its ability to inhibit uPA activity.

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