The evaluation of relationship between adiponectin levels and epicardial adipose tissue thickness with low cardiac risk in Gilbert`s syndrome: an observational study

Gilbert sendromlu hastalarda epikardiyal yağ doku kalınlığı ve adiponektin seviyesinin düşük kardiyak riskle birlikteliğinin değerlendirilmesi: Gözlemsel bir çalışma

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ABSTRACT

Objective: Atherosclerotic heart diseases are less frequently seen in patients with Gilbert's syndrome (GS). We aimed to investigate whether serum adiponectin (APN) and epicardial adipose tissue (EAT) thickness have an effect beside the antioxidant effect of bilirubin in lowering the incidence of the atherosclerotic process.

Methods: Sixty-eight patients diagnosed with GS (39 females and 29 males) who had applied at the internal medicine clinic of the hospital were included in this cross-sectional, observational study. The control group included 63 healthy people (39 females and 24 males). EAT thickness was measured by echocardiography. The serum APN levels were also checked. Statistical analysis was performed by using independent sample t-test, Pearson correlation and linear regression analyses.

Results: The mean age of the GS group was 28±9 years, and the average EAT thickness was found to be 2.5±0.1 mm. The mean age of the control group was 26±6 years, and the average EAT thickness was found to be 4.2±0.5 mm. When comparing the two groups, the EAT thickness of the GS group was found to be significantly lower ($p<0.001$) than that of the control group. In the GS group the APN was 14.9±4.2 mg/L, and in the control group the APN was 12.6±4.5 mg/L ($p<0.022$). We found that total bilirubin (β=-1,607, $p<0,001$) and indirect bilirubin (β=1,086, $p<0,001$) have an independent association with decreased EAT thickness.

Conclusion: EAT thickness is associated with coronary atherosclerosis. Low EAT thickness may be related with low release of proinflammatory cytokine. High levels of APN may be related high anti-inflammatory effect. Therefore, low EAT thickness and high levels of APN may demonstrate protective effect on atherosclerotic heart diseases in GS patients. *(Anadolu Kardiyol Derg 2013; 13: 791-6)*

Key words: Adiponectin, epicardial adipose tissue, Gilbert`s syndrome, bilirubin, regression analysis

ÖZET

Amaç: Gilbert sendromlu (GS) hastalarda aterosklerotik kalp hastalıkları daha az sıklıkla görülmektedir. Bilirubinin antioksidan etkisi yanında serum adiponektin (APN) ve epikardiyal yağ doku (EYD) kalınlığının aterosklerotik sürecin az görülmesinde etkisi olup olmadığını araştırmayı amaçladık.

Yöntemler: Hastanemiz iç hastalıkları polikliniğine başvuran toplam 68 GS hastası (39 kadın, 29 erkek) bu kesitsel gözlemsel çalışmaya dahil edildi. Kontrol grubuna 63 sağlıklı birey (39 kadın, 24 erkek) alındı. EYD kalınlığı ekokardiyografi ile ölçüldü. Serum APN seviyeleri de ölçüldü. İstatistiksel analizde bağımsız örneklem t-testi, Pearson korelasyon ve lineer regresyon analizi kullanıldı.

Bulgular: GS hastalarda ortalama yaş 28±9 yıl ve ortalama EYD kalınlığı 2,5±0,1 mm bulundu. Kontrol grubunun yaş ortalaması 26±6 yıl ve ortalama EYD kalınlığı 4,2±0,5 mm bulundu. İki grup karşılaştırıldığında GS grubunda EYD kalınlığı kontrol grubundan anlamlı düşük bulundu ($p<0,001$). GS grubunda APN 14.94 mg/L ve kontrol grubunda APN 12,64 mg/L idi ($p<0,022$). Total bilirubin (β=-1,607, $p<0,001$) ve indirekt bilirubin (β=1,086, $p<0,001$) ile azalmış EYD kalınlığı arasında bağımsız bir ilişki olduğunu bulduk.

Sonuç: EYD kalınlığı koroner ateroskleroz ile ilişkilidir. EYD kalınlığı azlığı pro-enflamatuvar sitokinlerin salınım azlığı ile ilişkili olabilir. APN seviyeleri yüksekliği ile yüksek anti-enflamatuvar etki ilişkili olabilir. Sonuç olarak EYD kalınlığı azlığı ve APN yüksek seviyesi GS hastalarında aterosklerotik kalp hastalığı üzerine bu parametrelerin koruyucu etkisi olduğunu gösterebilir. *(Anadolu Kardiyol Derg 2013; 13: 791-6)*

Anahtar kelimeler: Adiponektin, epikardiyal yağ doku, Gilbert, bilirubin, regresyon analizi

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Introduction

Adiponectin (APN) is an excellent adipokine with multiple beneficial effects such as anti-oxidative (1), anti-inflammatory (2), and antiapoptotic (3) activities in numerous organs and cells. Diminished plasma APN levels are commonly associated with all components of metabolic syndrome and atherosclerosis and are conversely correlated to visceral adipose tissue and may serve as a predictive factor for the syndrome (4). Visceral adipose tissue was found to be related to metabolic syndrome.

As reported in previous studies, adipose tissue is a complex endocrine organ that secretes a great number of molecules and hormones, which have systemic effects (5, 6). Increased epicardial adipose tissue (EAT) has been found to be related to increased ventricular mass (7). This tissue releases inflammatory cytokines (8). Additionally, reports indicate that an increased mass of pericardial adipose tissue is correlated with decreased APN secretion (9).

Gilbert's syndrome (GS) is an autosomal recessive disease; it is a benign condition that does not progress into chronic liver disease or fibrosis (10). GS occurs in 3%-17% of the population (12.4% males and 4.8% females) (11). Bilirubin glucuronidation is diminished owing to a partial defect in the UDP-glucuronosyl transferase enzyme (12). The syndrome shows a fluctuation of enhanced indirect bilirubin (IB) levels. However, it may not need lifelong treatment. It is diagnosed without giving any clinical manifestation.

GS lowers the incidence of atherosclerotic heart disease. This is related to the antioxidant effect of bilirubin (13). Oxidative stress and inflammatory processes play a role in the pathogenesis of atherosclerotic heart disease (14). The C-reactive protein (CRP) levels in patients with GS are lower than in healthy individuals (15). Lipid peroxidation plays a role in the pathogenesis of atherosclerosis (16). In patients with GS, low levels of CRP and reduced lipid peroxidation indicate low oxidative stress, a strong antioxidant capacity, and low inflammation processes. In fact, previous studies have shown IB to protect against lipid peroxidation, and act as a remover of peroxide radicals (17, 18). Consequently, it reduces the incidence of atherosclerotic heart disease. GS patients are known to have low inflammation and lipid peroxidation. They also have raised levels of IB, which is known to be an antioxidant and has cardio-protective effects.

There is no information on serum APN levels and EAT in patients with Gilbert`s syndrome.

We aimed to investigate the protective effect of bilirubin on plasma APN levels and EAT thickness in GS patients.

Methods

Study design

The present study was designed as a cross-sectional, observational study that was performed in Internal Medicine Department of medical Faculty of Recep Tayyip Erdoğan University between February and August 2012.

Study population

Sixty-eight patients diagnosed with GS (39 females, 29 males) who had applied to the clinic in the Recep Tayyip Erdoğan University Medical School Hospital were enrolled in the study. A control group of 63 healthy persons (39 females, 24 males), who were non-smokers and drinkers, were included. Both groups were between 16-45 years old. Two groups were evaluated clinically with echocardiography (ECHO) by a cardiologist and performing biochemical tests. Inclusion criteria were as follows: Increased levels of IB (≥0.8 mg/dL) with normal levels of lactate dehydrogenase (LDH) were not considered as hemolysis and corrected reticulocyte counts were obtained from reticulocyte smear of the patients and values lower than 2% were included in the study. Healthy subjects with IB <0.7 mg/dL who had not any known disease were included in the control group. Exclusion criteria for both groups were as follows: Having heart disease, chronic renal failure, diabetes mellitus, impaired fasting glucose, impaired glucose tolerance, hyperthyroidism, hypothyroidism, acute or chronic liver disease, cancer or any other chronic disease, acute or chronic infection, chronic drug usage, smoking, drinking alcohol, hypoalbuminemia, hematologic diseases such as myelodysplastic syndrome, leukemia, lymphoma and vitamin B12 deficiency. Increased levels of IB with elevated levels of LDH were considered as hemolysis and excluded from the study (19). Corrected reticulocyte counts were obtained from reticulocyte smear of the patients with normal levels of LDH, and the levels more than 2% were excluded (20).

The study was approved by the local ethics committees, and informed consent from each participant was obtained (Approval number of: 2012/20).

Study variables

The baseline characteristics of all patients including age, sex, body mass index (BMI), waist circumference (WC), systolic blood pressure, and diastolic blood pressure were recorded carefully. Biochemical blood tests (IB, total bilirubin (TB), fasting plasma glucose (FPG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP), lipid profiles, LDH, reticulocyte, hematologic parameters, hepatitis panel, vitamin B12, thyroid stimulating hormone (TSH), APN and EAT thickness were obtained from the entire study population on admission.

Measures of laboratory tests

The biochemical tests were performed with the *photometric* assays of the Abbott Architect C16000 analyzer (Abbott Diagnostics, USA), and the TSH and vitamin B12 tests were performed using the Chemiluminescent Microparticle Immunoassay (CMIA) method of the Abbott Architect I 2000 immunology analyzer (Abbott Diagnostics, USA).

The CRP test was performed with the nephelometric method of the Coulter Image 800 device, (Beckman Coulter, California, USA) and HBsAg, anti-HCV and anti-HIV were tested with the Roche Cobas E 601 microelisa device (Roche Diagnostics,

England). The hematologic tests were performed using the Abbott Cell Dyn Ruby analyzer (Abbott Diagnostics, USA).

The concentration of total APN was measured using the enzyme-linked immunosorbent assay (ELISA) method. We used a commercially available high-sensitivity human APN ELISA method (BioVendor Laboratory Medicine, Inc., Czech Republic). The procedure employed for the ELISA method was according to the instructions provided by the manufacturer. Absorbance was measured at a wavelength of 450 ηm using the ELISA reader. The levels of APN are presented as μg/mL. The intra-assay and inter-assay coefficient of variation were 3.3% and 6.2%, respectively. The limit of detection (LOD) for the APN assay was 0.47 ηg/mL (BioVendor Laboratory Medicine, Inc., Czech Republic).

Diagnosis of Gilbert`s syndrome

There is no indication for liver biopsy in patients with GS. If a biopsy is carried out, it will show normal liver tissues. The previous laboratory tests of patients who met the above criteria were reviewed, and the elevation of IB at least twice at different times was accepted as GS. Patients with elevated levels of IB, who had no previous laboratory results were called after 15 days for retesting and persistent elevation, were included in the study.

Echocardiography

GS patients and healthy controls underwent complete transthoracic examinations including two dimensional, color flow and pulsed Doppler, tissue Doppler imaging, and EAT measurement with a GE-Vingmed Vivid S5 (GE-Vingmed Ultrasound AS, Horten, Norway) using a 2.5-3.5 MHz transducer. All examinations were performed by an experienced cardiologist, oblivious to the patients' clinical information.

Evaluation of EAT thickness

EAT was evaluated on the free wall of the right ventricle from the parasternal long axis view, using aortic annulus as an anatomic reference. We preferred to measure EAT thickness on the area of above the right ventricle, since this area is known to have the thickest EAT layer. EAT, determined as an echo-free space between the pericardial layers on a 2-dimensional echocardiography, was measured perpendicularly in front of the right ventricular free wall at the end diastole (21, 22). We magnified each form image for better visualization and exact measurement of EAT thickness and measured the thickest point of the EAT in each cycle. To standardize the measuring axis, we used the aortic annulus as an anatomical reference. The measurement was performed at a point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus. The mean value comprising three cardiac cycles of each echocardiographic view was used for statistical analysis.

Statistical analysis

The data analysis was performed using the statistical software SPSS for Windows (version 13.1; SPSS, Chicago, IL, USA). The results are reported as the mean±SD. All the results were analyzed by applying the Kolmogorov-Smirnov method for the determination of normal and abnormal data distribution. Groups showed normal distribution. The statistical significance of the differences in all parameters between the GS group and the control group were analyzed using the independent sample t-test. The relationship between the variables was analyzed with the Pearson's correlation. The linear regression analysis was performed to determine the independent relationship between EAT thickness and APN levels and other potential confounding variables. The differences were considered significant at $p<0.05$.

Results

Baseline clinical and echocardiographic characteristics

The mean age of the GS group was 28±9 years, and the average EAT was found to be 2.5±0.1 mm. The mean age of the control group was 26±6 years, and the average EAT was found to be 4.2±0.5 mm. When comparing the two groups the EAT and diastolic blood pressure were found to be significantly lower ($p<0.001$ and $p\leq0.026$) than that of the control group. The patients demographic data, blood pressures and EAT measurements are shown in Table 1.

Laboratory values in studied groups

The APN level was higher in patients with GS than in the controls ($p=0.022$), as well as TB and IB were significantly higher in patients` group ($p=0.001$ for both). High-density lipoprotein (HDL) levels of the GS group were found to be significantly higher than those in the control group ($p=0.007$). Although not statistically significant triglyceride (TG), low-density lipoprotein (LDL) and CRP levels of the GS group were lower than those in the control group. The relevant data on the GS patients and the control group are shown in Table 2.

Relationship of adiponectin and EAT with clinical and laboratory variables in GS

There was negative correlation between EAT and TB ($r^2=0.292$, $p<0.001$), IB ($r^2=0.230$, $p<0.001$), and HDL ($r^2=0.074$,

Table 1. The clinical characteristics and EAT thickness of the groups

Variables	GS (n=68)	Control (n=63)	*p
Age, years	28±9	26±6	0.139
Sex, M/F, n	29/39	24/39	0.599
BMI, kg/m^2	22.7±4.6	21.7±3.4	0.171
WC, cm	80.5±13	78.0±9	0.213
Systolic BP, mm Hg	106±13	107±11	0.459
Diastolic BP, mm Hg	68±7	71±7	0.026
EAT thickness, mm	2.5±0.1	4.2±0.1	0.001

Values are presented as mean±SD and number
*Student's t- test for independent samples and Chi-square test
BMI - body mass index, BP - blood pressure, EAT - epicardial adipose tissue, F - female, M - male, WC - waist circumference

Table 2. The results of laboratory parameters of the two groups

Variables	GS (n=68)	Control (n=63)	**p
Adiponectin, mg/L*	14.9±4.2	12.6±4.5	0.022
FPG, 70-110 mg/dL	93±8	95±8	0.149
AST, 0-55 IU/L	17±4	17±5	0.652
ALT, 5-34 IU/L	17±12	15±10	0.376
BUN, 15-43 mg/dL	27±10	25±7	0.306
Creatinine, 0.6-1.1 mg/dL	0.70±0.1	0.76±0.1	0.052
TC, 0-199 mg/dL	167±27	164±22	0.588
TG, 0-149 mg/dL	80±37	82±28	0.698
HDL, 35-70 mg/dL	53±11	48±8	0.007
LDL, 0-130 mg/dL	97±22	99±20	0.551
TB, 0.2-1.2 mg/dL	2.1±0.9	0.7±0.2	0.001
IB, 0.1-0.7 mg/dL	1.5±0.9	0.4±0.2	0.001
CRP, 0-0.8 mg/dL	0.2±0.3	0.3±0.6	0.311

*For patients having BMI <25 kg/m^2; normal range: M 10.9±4 mg/L, F 13.6±5.4
Data are presented as mean±SD
**Independent sample t-test
ALT - alanine aminotransferase, AST - aspartate aminotransferase, BUN - blood urea nitrogen, CRP-C - reactive protein, F - female, FPG - fasting plasma glucose, HDL - high density lipoprotein, IB - indirect bilirubin, LDL - low density lipoprotein, M - male, TB - total bilirubin, TC - total cholesterol, TG - triglycerides

p=0.003). EAT positive correlated with among (r=0.312, p=0.001), BMI (r=0.415, p<0.001), and TG (r=0.265, p=0.005). There was a positive correlation between TB (r=0.192, p=0.041), IB (r=0.188, p=0.046) and APN. There was no correlation between APN and EAT (r^2=0.002, p=0.583).

Factors associated with EAT thickness

In linear regression analysis (Table 3) in which EAT thickness was taken as a dependent variable age, BMI, TB, IB, CRP, APN, LDL and TG were included as independent variables, we found that TB (β=-1.607, p<0.001) and IB (β=1.086, p<0.001) have an independent association with decreased EAT thickness.

Discussion

In the current study, the control group was selected to have similar age and BMI with the GS patients group. EAT thickness of GS patients was found to be significantly lower than the control group. Additionally the level of serum APN was significantly higher in the patients group. EAT was positively correlated with increase in BMI, and reduction in EAT was associated with increased levels of TB and IB. The increase of TB and IB was found to be positively correlated with APN.

EAT is a true visceral fat tissue, given the previous reports indicating a strong correlation between the tissue and abdominal visceral fat deposits (23). After the discovery of the secretion of inflammatory mediators by subcutaneous and EAT its clinical importance has been raised. It has been found that subcutaneous and EAT secrete more chemokines and cytokines (24-26). The pro-inflammatory cytokines studied in the previous study,

Table 3. Determinants of EAT thickness in GS patients: linear regression analysis (r^2=0.557, p<0.001)

Independent variables	*Beta regression coefficient	*p
Age	0.044	0.677
BMI	0.286	0.003
SBP	0.189	0.277
DBP	-0.053	0.742
FPG	0.017	0.827
TB	-1.607	0.001
IB	1.086	0.001
CRP	0.045	0.565
APN	0.061	0.432
TC	-0.609	0.477
LDL	0.486	0.528
TG	0.167	0.486
HDL	0.193	0.596

*Linear regression analysis
APN - adiponectin, BMI - body mass index, CRP-C - reactive protein, DBP - diastolic blood pressure, EAT - Epicardial adipose tissue, FPG - fasting plasma glucose, HDL - high density lipoprotein, IB - indirect bilirubin, LDL - low density lipoprotein, SBP - systolic blood pressure, TB - total bilirubin, TC - total cholesterol, TG - triglyceride

CRP and other pro-inflammatory cytokines; contribute to the process of plaque destabilization and plaque rupture (27, 28). The association of thoracic aorta atherosclerosis and coronary atherosclerosis with EAT was known (29, 30). In the previous study a relationship has been shown between the atherosclerosis of thoracic aorta and EAT thickness (29). According to the results of the current study, EAT thickness in GS patients was strongly lower than that of the control group. Therefore, atherosclerotic process may progress slowly in GS patients. In addition, the released pro-inflammatory cytokines may be low and, as a result, cardioprotective effects may occur. In this study the low EAT thickness in GS group and its relation with bilirubin may demonstrate the cardioprotective effect of bilirubin.

APN is an adipose tissue-derived hormone of interest to scientists studying diseases such as obesity, atherosclerosis, diabetes, and metabolic syndrome, to name a few (31). APN inhibits the production of pro-inflammatory cytokines and chemokines in endothelial cells, and inhibits their ability to become activated in response to various inflammatory stimuli. APN also attenuates the adverse effects of various proatherogenic mediators including cytokines and oxidized low-density lipoprotein (32). APN attenuates oxidized low-density lipoprotein and hyperglycemia-induced reactive oxygen species generation (33). In this study, APN was strongly higher in GS patients; as a result, cardioprotective effects may occur.

A previous study has reported that level of APN released from EAT local expression were lower in patients with hypertension (34) as well as lower levels of the hormone were related to the promotion of coronary artery disease (35). Another study has suggested that local expression of APN has local effect on myo-

cardium and coronary artery rather than systemic effects (36). Additionally low level of APN was found to be related with coronary artery disease. In our study serum APN levels were higher in GS group. In these patients the level of local expressed APN from EAT may be higher than healthy subjects and may contribute to the cardioprotective effect. However, in this study while there was a positive correlation between APN and bilirubin levels there was no correlation with EAT thickness. APN may be released more intensively from other adipose tissues. This study is a pilot study new studies are needed in this regard.

The protective effect of increased levels of bilirubin against atherosclerotic heart disease has been reported in many studies (17, 18). Bilirubin is known to be a potent antioxidant that decreases lipid peroxidation and the chronic inflammation process (37). Thus, it is known to possess a cardiovascular protective effect. In the study, although the levels of LDL and CRP were not strong significant, they were lower in the GS group. This finding shows that GS patients may have lower lipid peroxidation and inflammation. Thus, less oxidative stress may occur in GS patients. Levels of EAT in GS patients is lower than in healthy patients, so the release pro-inflammatory cytokines may be lower. As a result, this contributes to the formation of slower atherosclerotic processes. In the GS group, the presence of high APN levels, resulting in anti-inflammatory and anti-oxidative activity, may result in a slower atherosclerotic process in these patients. Moreover, APN is known to have an in vivo glucose and lipid lowering effect (38). This may be the reason for low lipid panels. As both APN and bilirubin have antioxidant and anti-inflammatory effects, so a synergistic effect may be observed in GS patients. Moreover, HDL, which is known as a cardioprotective was significantly higher in the GS group than the control group. High levels of HDL in GS patients may increase the cardioprotective effect.

This study has shown that GS patients have lower diastolic blood pressure. In addition, systolic blood pressure was one unit lower. Although strongly insignificant, it is with clinical importance. Tan et al. (39) reported impaired endothelium-dependent vasodilatation with response to changes in APN levels, suggesting APN as a link between adipose tissue and vasculature. In fact, some EAT-related cytokines, such as APN or free fatty acids, have been linked to hypertension, coronary artery disease, endothelial dysfunction, and sympathetic over activity. Elevated plasma fatty acid concentrations may also stimulate cardiac autonomic nervous system activity through an increase in plasma catecholamine concentrations (40). Besides increased release of APN and lower EAT, GS patients have low diastolic blood pressure. This may result in lower sympathetic activity, so fewer cardiac diseases may occur.

Study limitations

There are different imaging modalities such as magnetic resonance (MR) and computer tomography (CT) which are currently gold standard for measuring visceral adipose tissue (41). These however are expensive, and are not routinely carried out in a typical cardiac patient. Nonetheless, echocardiogram provides a relatively inexpensive means to measure and quantify an important component of EAT, the EAT part of the visceral adipose tissue, which may have similar influence on cardiovascular risk profile. The number of subjects in our study may be insufficient to represent the general population. This is only a pilot study, and further studies are needed on this subject. Additionally, there was no long-term follow up, so the study focused on possibility and likelihood, rather than the exact future results. Further studies are needed with long-term follow up in terms of monitoring EAT and APN levels in GS patients. We did not measure inflammatory markers such as high sensitive CRP and APN. However, this is an observational study and our main was to investigate the relation between EAT thickness and GS. This study would provide more significant information on the cardioprotective effects in GS patients.

Conclusion

EAT thickness was lower and serum APN levels were higher in the GS group compared with the control group. Low EAT thickness and high levels of APN may be related to the protective effect of bilirubin on atherosclerotic heart diseases in GS patients.

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