RESEARCH ARTICLE

Use of Oral Antidiabetic Drugs (Metformin and Pioglitazone) in Diabetic Patients with Breast Cancer: How Does It Effect on Serum Hif-1 Alpha and 8Ohdg Levels?

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Abstract

Objective: The aim was to investigate indicators related to DNA damage and cancer pathogenesis in Type II diabetes cases with breast cancer. It was planned to evaluate the relationship between these markers with oral antidiabetic drugs. Research Design and Methods: Fourty patients and 10 healthy individuals were included in the study. HIF-1α and 8-OHdG are examined in blood samples taken from these individuals with an ELISA Kit. Statistical analysis of data was performed with 95% confidence using Windows package program SPSS 15.0. Results: HIF-1α parameters were found to be meaningfully higher in the patient group than the controls in both pretreatment and posttreatment periods ($p<0.05$). No significant differences in terms of 8-OHdG between patients and controls. However, posttreatment serum HIF-1α ve 8-OHdG levels was found lower than pretreatment levels in patients receiving metformin, but not with pioglitazone. Conversely, serum 8-OHdG levels decreased significantly in these patients. When patients were evaluated according to the treatment groups (pioglitazone vs. metfformin) no significant differences in terms of serum HIF-1α and 8-OHdG levels between treatment groups. Conclusions: HIF-1α levels decreased significantly in the patient group receiving metformin. However, there was no significant difference in terms of HIF-1α levels in the patients receiving pioglitazone.

Keywords: Breast cancer - diabetes - 8-OhdG - HIF-1α levels

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Introduction

Type 2 diabetes is a well-known endocrine and metabolic disorder which has reached epidemic proportions worldwide and represents a serious public health concern. It is estimated that it will affect approximately 366 million people by 2030 (Rathmann et al., 2004). Although the underlying mechanisms of the pathogenesis of Type 2 diabetes still remain to be determined, oxidative stress has been shown to be responsible, at least in part, for the progression of Type 2 diabetes, and supported by increased oxidative damage to lipids and DNA and impaired antioxidative defence systems in these patients (Bonnefont-Rousselot et al., 2000; Rosen et al., 2001). Numerous evidences have indicated that 8-OHdG not only is a biomarker of generalized, cellular oxidative stress but might also be a risk factor for cancer, atherosclerosis and diabetes. The oxidative hydroxylation of guanine in the 8-position is the most frequent and most mutagenic lesion in nuclear DNA. Oxidative damage to DNA, reflected in the formation of 8-OHdG, is important mutagenesis and carcinogenesis (Wu al., 2004). Many authors have examined the relationship between oxidative stress and several diseases such as cancer, obesity, Type 1 diabetes (Bast et al., 2002; Martinez-Outschoorn et al., 2010; Samuni et al., 2010; Van et al., 2010), Type 2 diabetes (Fridly and LE et al., 2006; Arif et al., 2010).

Diabetes and cancer are two heterogeneous, multifactorial, severe, and chronic diseases. Epidemiological studies clearly indicate that the risk of several types of cancer (including pancreas, liver, breast, colorectal, urinary tract, and female reproductive organs) is increased in diabetic patients. Obesity, hyperglycemia, and increased oxidative stress may also contribute to increased cancer risk in diabetes (Paolo et al., 2009).

Diabetes is frequently associated with hypoxia and is known to impair ischemia-induced neovascularisation and other forms of adaptive cell and tissue responses to low oxygen levels. Hyperglycaemia appers to be the driving force of such deregulation. Recent data indicate that destabilisation of HIF-1 is most likely the event that transduces hyperglycaemia into the loss of the cellular response to hypoxia in most diabetic complications (Bento et al., 2011). HIFs are nuclear transcription factors and function as oxygen-sensitive α subunit and β heterodimers (ARNT). All isoforms of HIFα, HIF1α, HIF2α and HIF3α require the ubiquitously expressed subunit aryl hydrocarbon nuclear translocator (ARNT or HIF1β) as

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an obligate heterodimerization partner for activation of target genes. HIF function is primarily regulated by HIF1α protein stability.

Breast carcinoma, is the second leading cause of cancer deaths in women. Notably, breast cancer is the most common invasive cancer in women, and metastasis significantly contributes to the morbidity and mortality of breast cancer (Liang et al., 2004). A hypoxic microenvironment is crucial for tumor metastasis. HIF-1α acts as an important mediator of hypoxic response; significant associations between HIF-1α overexpression and patient mortality have been shown in cancers of the oligodendroglioma, breast, cervix, oropharynx, ovary, and endometrium (Zhong et al., 1999; Talks et al., 2000; Van et al., 2005). Ultimately, HIF-1 activates the expession of numerous genes that help cells to survive at low oxygen levels. Changes in gene expression directly or indirectly regulated by HIF-1 extend to more than 100 genes, which are involved in survival mechanisms, such as angiogenesis, cell growth, apoptosis.

There is so complicated relation between cancer and HIF-1α. (Kimbro et al., 2006). One of the most recently identified pathways modulating and influencing HIF-1α regulation is the RAS-ERK pathway (Lim et al., 2004). Several investigators have recently shown that RAS effects VEGF expression through HIF-1α. Moreover, mTOR and AMPK signaling pathways play a role in the pathogenesis of cancer. m TOR is a serin-threonine protein kinase that belongs to the PIKK (phophoinositide 3-kinase (PI3K)-related kinase) family (Hay et al., 2004; Wullschleger et al., 2006). mTOR is up-regulated in many cancer cells as a result of genetic alterations or aberrant activation of the components of PI3-k/Akt pathway, contributing to dysregulation of cell proliferation, growth, differentiation and survival (Feng et al., 2005; Sabatini, 2006). AMPK is a heterotrimeric complex comprising a catalytic α subunit and regulatory β and α subunit (Hardie et al., 2001). AMPK is switched on during situations in which the cellular level of ATP is depleted and the level of AMP is increased, such as those triggered by hypoxia, glucose deprivation, oxsidative stress, tissue ischemia. These are all recognised cellular stresses that reduce cellular ATP and elevate AMP levels causing AMPK activation (Kemp et al., 2003).

Metformin has been widely used for treating type 2 diabetes without the stimulation of insulin production, and for this reason, it is considered an insulin sensitizer (Bailey et al., 1996). The UK Prospective Diabetes Study (UKPDS) showed that metformin could reduce macrovascular morbidity and mortality (UK Prospective Diabetes Study, 1998), suggesting that it achieved its antiatherogenic and anti-inflammatory effects by means of antioxidant properties (Abbasi et al., 2004).

Advances in our understanding of AMPK and targeted mTOR therapy have become clinically relevant given the association between a lower incidence of cancer and metformin usage in diabetic patients (Evans et al., 2005). Experimental studies on the effects of metformin on epithelial cells have demonstrated that activation of the AMPK pathway by metformin reduces cellular proliferation and mTOR activation (Hawley et al., 2002).

The thiazolidinediones (TZDs) bind to the γ subtype of peroxisome proliferator-activated receptors (PPARs). Pioglitazone is a PPARγ ligand used in the treatment of type 2 diabetes. It is indicated as an adjunct to diet and exercise to improve glycemic control. It is generally not used as a first-line therapy (Nathan et al., 2009). A link between pioglitazone and bladder cancer first appeared in preclinical studies and was first reported on the U.S. pioglitazone label in 1999, but experimental studies recently suggested that it might be a rat-specific phenomenon (Suzuki et al., 2010). PPAR-γ agonists have been shown to induce apoptosis in several malignant cell lines (Elstner et al., 1998) and to inhibit the invasive activity of colon and breast cancer cells (Liu et al., 2003). In contrast, animal toxicity studies have suggested a possible increased cancer risk in multiple organs in association with a wide variety of PPAR-γ and dual PPARα/γ agonists (Rubenstrunk et al., 2007).

In this study, It was planned investigation in terms of 8-OHdG levels and HIF-1α levels in women patients with breast cancer and newly diagnosed type 2 diabetes. These parameters were evaluated among healthy volunteers and patients (pretreatment/posttreatment).

Materials and Methods

The study included 40 female patients with breast cancer. They were accepted to Department of Medical Oncology of Izmir Ataturk Training and Research Hospital. They were consulted to Department of Endocrinology in terms of diabetes diagnosis. Thus, It was choosen the patients diagnosed newly type 2 diabetes (ADA Guidelines 2012). The study protocol was approved by the Ethical Committee of Katip Celebi University Hospital. Written informed consent was taken from all subjects before participating in the study. It was proposed diet, lifesyle modification besides oral antidiabetic agents. The patients were randomized for oral anti diabetic treatment (metformin n=20 vs pioglitazone n=20). It was suggested 2000 mg and 15 mg daily dose for oral antidiabetic agents (respectively; metformin and pioglitazone). Blood samples were collected in the first visit (pretreatment) and second visit (3 months after initiation of therapy). 8-OHdG and HIF-1α levels of patients and control subjects were evaluated by ELİZA kit.

Measurements of blood glucose levels and HbA1c

Blood glucose and HbA1c levels were measured at South West Pathology. Blood glucose levels was assessed using fasting blood samples obtained through venipunture and HbA1c was measured using HPLC.

In vitro Quantitative Determination of Human HIF-1 Concentrations

Human HIF-1 ELISA Kit is applied to serum samples of subjects. This test is worked with sandwich based ELISA method. HIF-1 alpha protein levels were quantified using the Human HIF-1 Alpha ELISA kit (Cusabio Biotech, Chine). Procedure was as described in the protocol provided by the manufacturer. Absorbance

was measured at 450 nm using an Microplate Reader Multiscan FC (Thermo-Scientific). The HIF-1 alpha protein concentration in each well was calculated using the equation of the HIF-1 alpha standard.

Determination of serum 8-OHdG levels

Serum 8-OHdG was measured using an ELİZA Kit, Cayman Chemical, USA. The test utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8-OHdG conjugate. This format has the advantage of providing low variability and increased sensivity compared with assays that utilize an antigen-coated plate. Procedure was as described in the protocol provided by the manufacturer. Absorbance was measured at 450 nm using an Microplate Reader Multiscan FC (Thermo-Scientific). The results were calculated with the Cayman data analysis system.

Statistical Analysis

Statistical analysis of data was performed with 95% confidence by Windows package program SPSS 15.0. All variables were summarized in the tables. Mann Whitney U test was performed for data comparison between groups. Wilcoxon Signed Ranks test was used for comparison between the first and second measurements. $p < 0.05$ value was considered statistically significant.

Results

The mean age of the patients and healthy volunteers were similar. There was not statisticaly significant diffrence in terms of mean age between treatment groups. In our study, there was statisticaly significant difference in terms of HIF-1α levels between patients and control group. Conversely, It was not detected significant diffrence in terms of 8-OHdG levels between these groups (Table 1). It was shown pretreatment and posttreatment serum markers of the patients receiving oral anti diabetic agent (metformin vs pioglitazone) in Table 2. In our study, HIF-1α levels after metformin treatment was lower compared to pretreatment. Conversely, there was not significant difference in terms of HIF-1α levels after pioglitazone treatment. Furthermore, It was found statisticaly significant difference in terms of 8-OHdG levels after metformin and pioglitazone treatment. ($p<0.05$).

Table 1. The Evaluation of Serum HIF-1α and 8-OHdG Levels in Subjects

	Patient (n=40)	Control (n=10)	p value
HIF-1 α (pmol/μL) (mean±SD)	1.41±0.48	0.60±0.23	0.001
8-OHdG (ng/ml) (mean±SD)	0.26±0.03	0.27±0.03	0.085

Table 2. The Analysis of Serum Levels HIF-1α and 8-OHdG According to the Treatment Groups

Metformin Group (n=20)	p value	*Pioglitazone* Group (n=20)	p value
First HIF-1α measurement (pmol/μL) (mean±SD)			
1.431±0.39	0.032	1.388±0.56	0.093
Second HIF-1α measurement (pmol/μL) (mean±SD)			
1.251±0.41	0.032	1.181±0.49	0.093
First 8-OHdG measurement (ng/ml) (mean±SD)			
0.262±0.034	0.001	0.262±0.030	0.036
Second 8-OHdG measurement (ng/ml) (mean±SD)			
0.243±0.025	0.001	0.251±0.025	0.036

Discussion

Diabetes mellitus (DM) is a serious and growing health problem worldwide and is associated with severe acute and chronic complications that negatively influence both the quality of life and survival of affected individuals. Diabetes and cancer have a complex relationship that requires more clinical attention and well-designed studies. A series of recent studies and meta-analyses confirm that the risk for several solid and hematologic malignancies is elevated in diabetic patients. The risk of cancers of female reproductive organs is also increased in DM. Both breast and endometrial cancer risks are increased in diabetic women. Several biological mechanisms may be involved, mostly regarding sex hormone abnormalities. Hyperinsulinemia may increase the levels of bioactive estrogens by decreasing the concentration of circulating sex hormone-binding globulin. A positive association between breast cancer mortality and diabetes was found in some studies (Paolo et al., 2009).

The oxidative hydroxylation of guanine in the 8-position is the most frequent and most mutagenic lesion in nuclear DNA. Oxidative damage to DNA, reflected in the formation of 8-OHdG, is important mutagenesis and carcinogenesis (Lily et al., 2004). In breast cancer, higher levels of 8-OHdG in DNA were detected in both cancer tissues and blood cells. Musarrat et al. has shown earlier that the accumulation of 8-OHdG in nuclear DNA had predictive significance for breast cancer risk assessment (Musarrat et al., 1996). It is widely postulated that the etiology of Type 2 diabetes has a strong association with oxidative stres (Rahimi et al., 2005). However, a number of studies on the role of oxidative stress in Type diabetes have shown inconsistent results (Bhatia et al., 2003), and this discrepancy may exist with respect to patient age, duration of diabetes, treatment methods, techniques used to measure oxidative stress etc. Several studies have shown a lack of association between diabetes and increased DNA damage level (Anderson et al., 1998; Hannon-Flecther et al., 2000). It was reported that glucose excursions in subjects with Type 2 diabetes trigger the activation of oxidative stress (Zeng et al., 2010). Al-Aubaidy emphasized that serum 8-OHdG is an early oxidative stres marker in the patients with pre-diabetes and Type 2 diabetes (Al-Aubaidy et al., 2010). In this study, It was not found difference in terms of 8-OHdG levels in between the patients and control subjects. Serum 8-OHdG levels were detected low compared to pre-treatment ($p<0,05$).

HIF-1 activates the expession of numerous genes that help cells to survive at low oxygen levels. Changes in gene expression directly or indirectly regulated by HIF-1 extend to more than 100 genes, which are involved in survival mechanisms, such as angiogenesis, cell growth, apoptosis. Since HIF-1 is a master regulator of the cellular response to hypoxia, it is not surprising that HIF-1 deregulation is directly associated with the loss of cellular adaptation to low oxygen in diabetes. In some studies shown that

changes in the function of HIF-1 can contribute to the development of diabetes (Gunton et al., 2005). These observations highlight a critical link between diabetes and the HIF-1 pathway.

Breast carcinoma, is the second leading cause of cancer deaths in women. A hypoxic microenvironment is crucial for tumor metastasis. HIF-1α acts as an important mediator of hypoxic response; significant associations between HIF-1α and patient mortality (Talks et al., 2000). HIF-1α promotes multiple steps of the metastasis program (Semenza et al., 2010). HIF-1α was associated with poor prognosis in locally advanced breast cancer patients (Luiz et al., 2011). In a study, the measurement of HIF-1α was not associated with clinical tumor grading or tumor size (Bos et al., 2001). In our study, HIF-1α levels in diabetic patients with breast cancer were found to be significantly higher than in healthy controls ($p<0.05$).

Inhibition of HIF1 in adipose tissue ameliorates obesity and insulin resistance. It was thought that HIF1 could provide a novel potential therapeutic target for Type 2 diabetes (Changtao et al., 2011). When taken together, the associations of HIF-1α and proliferation markers, enhanced expression of growth receptors and the the increase glycolic activity supports the role of HIF-1α in breast cancers. It also opens the possibility for targeting HIF-1 with combination therapies to affect the proliferation rate of breast cancers.

Advances in our understanding of AMPK and targeted mTOR therapy have become clinically relevant given the association between a lower incidence of cancer and metformin usage in diabetic patients (Evans et al., 2005). In addition, given that obesity, insulin resistance may play a role in the onset of malignancies including breast cancer, endometrial cancer, prostate cancer (Giovannucci et al., 1995; Del et al., 1998; Gapstur et al., 2001) Metformin users have a low incidence of cancer. Experimental studies on the effects of metformin on epithelial cells have demonstrated that activation of the AMPK pathway by metformin reduces cellular proliferation and m TOR activation (Hawley et al., 2002). Some authors reported that metformin use was associated with lower risk of cancer of the colon or pancreas, but did not affect the risk of breast or prostate cancer (Currie et al., 2009). In our study, It was shown that metformin therapy in patients with breast cancer reduced serum levels of HIF-1α ($p<0.05$).

Pioglitazone is a PPARγ ligand used in the treatment of type 2 diabetes. In the large PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study, 14 bladder cancers occured in the pioglitazone arm (0.5%) versus 6 in the placebo arm (0.2%), (Dormandy et al., 2005) and in September 2010, the U.S. Food and Drug Administration (FDA) announced an ongoing investigation on the possible risk in humans (Food and Drug Administration, 2010). Recent studies have demonstrated that peroxisome proliferator activator-receptors (PPAR)-γ is expressed in various cancer tissues and its ligand induces growth arrest of cancer cell through apoptosis (Rikio et al., 2003). Some reports demonstrate that PPAR-γ ligands inhibit growth of cancer cells in vitro and in vivo (Kubota et al., 1998). PPAR-γ, a nuclear hormone receptor, provides a strong link between lipid metabolism and the regulation of gene transcription. PPAR-γ acts in the adipose tissue and promotes lipogenesis under anabolic conditions. Recently, the receptor has also been implicated in inflammation and tumorigenesis. Significant evidence from many experimental systems suggest that PPAR-γ is important in carcinogenesis. PPAR-γ is upregulated in malignant tissue, and PPAR-γ ligands induce terminal differantiation in human breast, colon and lung cancer cells (Mueller et al., 1998). Some authors found no clear evidence of an association between use of pioglitazone and risk of the incident cancers examined (Assiamira et al., 2011). Clinical trial and epidemiologic data on TZDs and cancer risk are limited and results from the few studies conducted to date have been conflicting (Govindarajan et al., 2007; Koro et al., 2007). Some authors reported that short-term use of pioglitazone was not associated with an increased incidence of bladder cancer, but use for more than 2 years was weakly associated with increased risk (James et al., 2011). In our study, HIF-1α levels of patients treated with pioglitazone did not increase. This situation may be related to follow-up period. Ultimately, pioglitazone is contraindicated in patients with current bladder cancer or a history of bladder cancer according to European Medicines Agency, 2011 (European Medicines Agency, 2011). However, long-term large-scale studies in patients with other types of cancer are needed.

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