INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by a pre-diabetic phase of varying duration, where increasing aberrant glucose control can be demonstrated by a variety of biochemical tests and procedures, chiefly the random and fasting blood glucose levels, the oral glucose tolerance test, and where available, tests for demonstrating insulin resistance. This latter phenomenon is in fact the underlying basis for the abnormal glucose control, preventing glucose and other metabolites from entering body cells, manifesting as raised blood glucose levels because of its action on skeletal muscle. This lack of insulin effect has been called the Insulin Resistance Syndrome (IRS), which are pre-diabetic lesion and the hallmark of fully developed type 2 diabetes mellitus. This study aims at identifying the abnormal status of the PPAR-γ in adipose tissues of type 2 diabetes mellitus patients, when compared with matched normal controls. Methods: This cross-sectional study was conducted in Ayub Medical College, Abbottabad, from 2012 to 2014. Sample included three equal groups of patients. Group-1 with diagnosed type 2 diabetes mellitus, aged 40–65 years, acting as the test group, Group-2 included non-diabetic obese, and Group-3 with normal subjects. Transcription Factor Assay for Peroxisome Proliferator Activated Receptor Gamma (gamma PPAR) was done on ELISA Technique from Nuclear Extract procured from Adipose Tissue of the subjects. Results: Mean age of enrolled participants was 48.93 SD±6.32 years. Patients ranged between ages of 40 years to 67 years. The mean values of PPAR in normal, obese and diabetic group were 1.72 SD±0.28, 1.282 SE±0.18 and 1.283 SE±0.18 respectively. The difference in mean values of PPAR was significant p<0.05. Conclusion: The levels of PPAR-γ in patients with type 2 Diabetes Mellitus and Obese cases are significantly lower than normal controls. Keywords: Type 2 diabetes mellitus, PPAR, insulin resistance, receptors

ORIGINAL ARTICLE

EXPRESSION OF PEROXISOME-PROLIFERATOR ACTIVATED RECEPTORS-γ IN DIABETICS, OBESE AND NORMAL SUBJECTS

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Background: Current research in type 2 diabetes mellitus focuses on the role of Peroxisome-Proliferator Activated Receptors (PPARs) in the pathogenesis of the Insulin Resistance Syndrome (IRS), which are pre-diabetic lesion and the hallmark of fully developed type 2 diabetes mellitus. Although some basic questions have been resolved, much remains to be studied, particularly in view of possible therapeutic intervention. A promising approach is the use of PPAR agonists like Thiazolidinedione (TZD) and related compounds, which act upon the receptor and cause restoration of cell function like increased uptake of glucose, increased oxidation of FFAs and lipids, even return of blood pressures to normal. Most of the research work has been performed on adipose tissue and skeletal muscle, as these are the major tissues involved in Insulin-dependent glucose uptake and lipid utilization. In fact, use of TZD has been mostly associated with beneficial effects on blood glucose levels because of its action on skeletal muscle cells. PPAR activity has also been documented in vascular endothelium including coronary endothelium, and may be involved in some kind of endothelial injury predisposing to coronary artery events, like angina and atherosclerotic narrowing, perhaps even in thrombosis.

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It has been shown that increased activity of these PPAR receptors leads to obesity, whereas decreased activity leads to the IRS.\(^6\) Thus the implications for diabetes are obvious and of great clinical significance, even though the entire sequence of events has not yet been worked out. These receptors are prime triggers for adipocyte differentiation and cause the deposition and storage of fat in newly formed adipose cells. They also cause the differentiation of fibroblasts to adipose cells.\(^9\)

PPAR-\(\gamma\) receptors appear to be a two-edged sword, so that their increased expression is also both beneficial and harmful, and their loss-of-function is also both harmful and useful in some instances. These receptors thus appear to be prime targets for further research aimed at defining precisely the balanced state that they exist in normally, and the triggers that upset the balance in either direction to cause disease.\(^10\)

This study was conducted to determine the status of the PPAR-\(\gamma\) in patients with and type 2 Diabetes Mellitus and compare the results with obese and normal matching controls.

**MATERIAL AND METHODS**

The cross-sectional study was carried out in the Department of Chemical Pathology, Ayub Medical College, Abbottabad and Department of Biochemistry, Faculty of Health Sciences, Hazara University, Mansehra from 2012 to 2014. A total of 105 subjects were included in the study. Sample included three equal groups of patients. Group-1 with diagnosed type 2 diabetes mellitus, aged 40–65 years, acting as the test group, group-2 included matched non-diabetic obese and group-3 with matched normal subjects. Sampling was non-probability purposive sampling, as the study duration does not allow random sampling. Patients of either sex between the ages of 40–65 years were included in this study. Subjects were selected on convenient basis from surgical ward of the Ayub Teaching Hospital Abbottabad, undergoing elective surgical cases not having any derangement in liver or renal functions. Fasting and random blood glucose levels were determined to rule out diabetes among non-diabetic patients. All baseline line investigations were performed to rule out any concurrent disease. PPAR-\(\gamma\) status was assessed by using transcription factor assay on PPAR-\(\gamma\) receptors in sections of the above-mentioned tissues of patients and controls. Quantification of gamma PPAR was done by transcription factor assay by ELISA on DAS plate reader, to enable a comparison of PPAR-\(\gamma\) expression between patients and controls. All data were entered in pre-designed pro forma and analysed using SPSS-17.

**RESULTS**

This research enrolled 105 patients to determine the status of the PPAR-\(\gamma\) in patients who type 2 Diabetes Mellitus, obese and compare the results with normal matching controls. Thirty-five patients each were enrolled in these three groups

Mean age of enrolled participants was 48.93 SD±6.52.years. Patients ranged between ages of 40 years to 67 years. Most of the participants (74%) belonged to ages between 40 to 50 years. Of the enrolled subjects, 50 (47.6%) were male and 55 (52.4%) were female. Most of these subjects 94 (89.6%) belonged to district Abbottabad followed by 5 (4.8%) from district Mansehra.

Study participants which were divided in three groups of Diabetic, Obese and Normal and were compared for demographic and Biochemical profiles. The three groups were similar in gender; age group and socio economic status with no statistical significant difference between the groups was observed with respect to above mentioned demographic variables.

Levels of Gamma PPAR measured showed most of the readings were below normal value of 1.75\(\mu\)gm/\(\mu\)l. 82.9% (87) of the subjects had Gamma PPAR levels below normal. Only 17% (18) participants had Gamma PPAR values above > 1.75 \(\mu\)g/\(\mu\)l which is considered as normal. The mean value of Gamma PPAR observed was 1.43 \(\mu\)g/\(\mu\)l SD±0.3

 Gamma PPAR values was more than 1.75mmol (normal values) in 16.7% of the participants assigned to normal group and the mean value of PPAR in same group was 1.72 SD±0.28. Whereas PPAR values in obese and diabetics were below normal value of 1.75mmol in 97% of participants in both groups. The mean values of PPAR in obese and diabetic group were 1.282 SD±0.18 and 1.283 SD±0.18 respectively. The difference in mean values of PPAR was significant \(p<0.05\). The low mean value of PPAR in obese and diabetic was suggestive of low number of Gamma PPAR nuclear receptors. Inter group variation for values of PPAR between normal and obese and diabetic group was significant \(p<0.05\), as summarized in table-1 and figure-1.

**Table-1: Comparison of PPAR levels among the groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>≤1.25</th>
<th>1.26–1.5</th>
<th>1.51–1.75</th>
<th>1.76–2.00</th>
<th>2.1 and above</th>
<th>(p)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>8</td>
<td>10</td>
<td>6</td>
<td>35</td>
<td>2.5%</td>
<td>27.0%</td>
</tr>
<tr>
<td>Obese</td>
<td>19</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>47.5%</td>
<td>40.5%</td>
</tr>
<tr>
<td>Type-2 DM</td>
<td>20</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>50.0%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>37</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

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DISCUSSION

The World Health Organization (WHO) estimates that the global prevalence of diabetes in all age groups would rise from 2.8% to 4.4% by 2030. This means that the persons effected by diabetes would be doubled, i.e., from 171 million to 366 million.\(^1\)

The International Diabetes Federation (IDF) has projected similar statistics. The federation predicts that the numbers in adults (20 years and over) would be increasing from 194 million in 2003 to 333 million by 2025.\(^2\)

Asian populations are unique in a sense that they are multiracial and they have a multi-facet causes that predispose the population to type 2 diabetes, and over that the factors predisposing the population in Asia to are complex and varied among the population members. The major cause of type-2 diabetes are either or both; impaired insulin secretion and impaired insulin action. This gets aggravated by the higher degree of glucotoxicity. These components may have genetic inclination in the effected population. And in the vicious cycle of diabetes pathogenesis, Lipotoxicity also plays a pivotal role in causing insulin resistance and ß-cell damage.\(^3\) In the pathogenesis of diabetes, ß-cell physiology undergoes a series of changes. With on-going obesity, lipotoxicity and impaired glucose metabolism ß cells respond with compensatory increase in insulin secretion. Such changes are seen even in non-diabetics who have a strong family history of diabetes. With progressive insults on the glucose metabolism, the ß-cell function declines and insulin-to-glucose ratio diminishes, and this ultimately leads to decompensation phase with overt expression of clinical manifestation of diabetes. Asians are found to be more inclined to insulin resistance than people living in other parts of the world. This Insulin resistance and compensatory increase in insulin secretion is even reported in children and adolescents from Asian Indian origin.\(^4,5\) All these factors probably play a major role in the escalating numbers of diabetes in young populations in Asia over the current decades.\(^4\)

The condition known as pre-diabetes increases the risk of developing type-2 Diabetes 3–10 fold. And hence, it is significant in diagnosis and its significant economic outcomes and associated disease burden, and preventing diabetes. There is lack of specific practice recommendations for dealing with pre-diabetes, sound interventions, both pharmacological and life-style modifications can play important roles in the prevention of diabetes and hence can play vital role in obviating the long-term complications.\(^6\)

Also, pre-diabetes also poses risk for development of microvascular and macro-vascular complications. These complications, especially the microvascular complications are small in pre-diabetes phase, but once diabetes sets in, these complications have strong footings, and then these worsens with poor glycaemic control.\(^7\)

The Diabetes Prevention Program (DPP), (Diabetes Prevention Program Research, 2002) studied the role of Troglitazone in Prevention of Diabetes (TRIPOD),\(^8\) ACT NOW for the Prevention of Diabetes (ACT NOW), Diabetes Reduction Assessment with Ramipril and Rosiglitazone (DREAM), and the Study To Prevent Non-Insulin Dependent Diabetes (STOP-NIDDM). All these studies aimed to investigate the effects of drugs, such as biguanide, thiazolidinediones (TZDs), and alpha-glucosidase inhibitors.\(^9,10\)

At the crossroads of obesity, insulin resistance and long-term complications of diabetes including cardiovascular disease is the nuclear receptor PPARγ. This receptor is required for adipose tissue formation and it is also a target of insulin-sensitizing drugs for treating diabetes.\(^11\) PPARγ is the nuclear receptor family that includes 48 human transcription factors and is regulated by direct binding of steroid and thyroid hormones, vitamins, lipid metabolites, and xenobiotics.\(^12\)

The binding of PPARγ to the DNA sequences requires heterodimerization with another member of the nuclear receptor known retinoic X receptor (RXR). The binding of agonist ligands to PPARγ then initiates a conformation change. This then attracts transcriptional coactivators, including members of the steroid receptor coactivator (SRC) family.\(^13\)

Fat cells are known to develop from a fibroblast-like pre-adipocyte to a mature adipocyte which is loaded by fats.\(^14\) The pivotal role of PPARγ in proliferation of mature adipocytes is clearly understood by functional and genetic knock-down
experiments. PPARγ is also known to play an important role in regulating lipid metabolism in mature adipocytes. Most of these facts about PPARγ, and other physiological effects were highlighted after the discovery that thiazolidinediones (TZD) antidiabetic drugs are high-affinity agonist ligands for PPARγ. A potential mechanism that could result in improved insulin sensitivity is suggested by studies showing that treatment with PPAR-g agonists induces adipogenesis in vivo in the obese Zucker fa/fa rat model. That is supplemented by the fact that mutations resulting in loss of function of PPAR-g have been identified as the under lying pathology in the development of insulin resistance. In our study the level of PPAR receptors were significantly reduced in the obese and diabetic patients as compared to the normal subjects. This clearly explains and augments the use of PPARγ agonists, such as the thiazolidinediones, act as insulin sensitizers and improve insulin resistance in patients with T2DM. We identified higher levels even in obese patients, it is likely that PPARγ2 gene polymorphism and/or the genetically determined insulin resistance may be associated with residual C-peptide secretion and involve excessive BMI in type 1 diabetes.

The results of our study show higher level of PPAR expression in normal subjects as compared to the diabetics. Experiments with mice in which the PPARα gene has been deleted have been very illuminating. The PPARα null mice are without overt symptoms when fed ad libitum, except for moderately elevated plasma triglycerides levels, they demonstrate the metabolic abnormalities upon starvation. This results in elevated plasma free fatty acid levels, hypoketonemia, hypoglycaemia, elevated plasma urea levels, hypothermia, a decreased metabolic rate, and a fatty liver. Detailed mRNA and protein expression analysis of PPARα devoid mice demonstrated that the metabolic abnormalities are because of altered expression levels of a range of metabolic enzymes. Obesity has been known to be a direct indicator of fatty liver. The extent of accumulation of fat in liver occurs because of imbalance between fatty acid uptake, oxidation, synthesis, and esterification, and triglyceride secretion. Any imbalance in activity of these pathways has a major impact on fat accumulation in the liver. Among the several transcription factors are involved in the regulation of the above-mentioned pathways, most notably the sterol regulatory element binding protein 1 (SREBP-1) and PPARγ. This role of PPAR in fat storage in liver suggests that pharmacological activation of these transcription factors may be effective reduce liver steatosis. This role of PPAR receptors in lipid metabolism has been used pharmacologically as PPAR agonists in diabetics as well as in obese patients.

Our study showed that PPAR receptors were significantly reduced in diabetic patients, this is in accord with the findings of the other studies. Clinical trials in pre-diabetic and diabetic persons failed to demonstrate an effect of PPARα agonists on plasma glucose and insulin levels. Only for PPARγ there are convincing results demonstrating that PPARγ activation improves insulin sensitivity.

CONCLUSION

The levels of PPAR-γ in patients with Type 2 Diabetes Mellitus and obese cases are significantly lower than normal controls.

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