Role of Sub Clinical Hypothyroidism in Association with Adiponectin Levels Causing Insulin Resistance in Metabolic Syndrome: A Case Control Study

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Introduction: Metabolic Syndrome (Met S) is reported to be associated with sub clinical hypothyroidism (SCH). The aim of our study is to evaluate the role of SCH in association with adiponectin levels causing insulin resistance in metabolic syndrome.

Materials and Method: We recruited 100 study subjects; out of which 50 were cases of Met S, which were further divided into two groups based on presence and absence of SCH and 50 were healthy controls. Serum insulin, serum T_3, T_4, TSH were measured by chemiluminisence based immunoassay and serum adiponectin was measured by ELISA.

Results: Mean TSH levels were significantly higher in Met S cases as compare to control. Out of 50 cases of Met S, 22 (44 %) had SCH. Mean serum adiponectin were significantly lower in Met S cases as compare to control. On Pearson’s correlation analysis, TSH showed significant positive correlation with HOMA-IR and negative correlation with adiponectin levels. Strong association was found on the likelihood of low levels of adiponectin in Met S cases.

Conclusions: Met S cases showed insulin resistance and underlying SCH. SCH in Met S may cause altered adipocytes physiology which is associated with decreased release of insulin sensitising adiponectin which may lead to insulin resistance and future development of type II DM and associated co morbidities. Therefore, Met S cases should be screened for SCH and adiponectin levels thereafter. Also, our recommendation is SCH should be treated appropriately to attenuate insulin resistance and development of type II DM in Met S.

Key words: Metabolic Syndrome, Sub clinical Hypothyroidism, Adiponectin, Insulin Resistance, Adipocytes

INTRODUCTION

Metabolic syndrome (Met S) is a collection of cardiometabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia. This clustering of risk factors is associated with an increased risk of developing type II diabetes mellitus and cardiovascular disease [1, 2].

It has been seen that nearly 1.4 billion people are suffering from Met S because obesity is reaching an epidemic proportion worldwide [3]. The International Diabetic Federation (IDF) estimates that 25 % of the world’s population has Met S [4]. In western population the prevalence of Met S is around 20% in adults [5]. In India the prevalence of Met S is around 23.2% of the study population according to the WHO definition, 18.3% according to the NCEP-ATP III definition and 25.8% by the IDF definition [6].

The rise in the incidence of Met S and its complications has led researchers to look for pathophysiologial aspect of adipose tissue. Adipose tissue is a major endocrine and signalling organ, which secretes various chemical messengers known as adipokines [7]. Increase in the secretion of a number of pro-inflammatory and prothrombotic adipokines and decrease in anti-inflammatory adipokines such as adiponectin occurs in obesity, Met S and associated co-morbidities [8]. In this regard adiponectin is important as it is an insulin sensitizing adipokine [9].

In many epidemiological studies, thyroid dysfunction in the form of sub clinical hypothyroidism (SCH) has been found to be associated with Met S [10]. Researcher reported that thyroid hormone is essential for normal physiological function of adipocytes in the form of growth and differentiation of adipocytes. Normal thyroid function is associated with the modulation of adipokine release from adipocytes [11]. Therefore, any alteration in thyroid function may lead to abnormalities in the homeostasis of adipokines and thus associated comorbidities like type II DM in Met S [12]. The exact mechanism which links the thyroid abnormalities, type II DM and Met S is still unknown.

Presently, there is no universally accepted guideline for the management of SCH [13]. Even the adverse outcome due to SCH is underdetermined. In cases of Met S this becomes important as it may lead to insulin resistance and early development of type II DM. Development of type II DM is a known compli-
cation in both, Met S and hypothyroidism. There are no studies regarding biochemical markers to assess for early stages of insulin resistance in Met S patients with SCH. Also, in previous epidemiological and experimental studies, conflicting results of association between thyroid hormones and adiponectin levels have been reported [14]. So, the present study would help to understand pathophysiology in Met S patients with regards to SCH.

Adiponectin might be one of the many missing links causing insulin resistance in Met S patients under the setting of thyroid dysfunction in the form of SCH. Therefore, our study made an attempt to explore the cross talk between thyroid gland dysfunction and adipocytes in Met S which may lead to insulin resistance and hence future development of type II DM. We hypothesized that under subclinical hypothyroidism, adipocytes releases less insulin sensitising adipokine – adiponectin which may lead to insulin resistance in Met S.

MATERIALS AND METHODS

Study Design

This study is a hospital based case control study carried out at the department of Biochemistry and Medicine, Lady Hardinge Medical College, New Delhi from 2012 to 2016. Research approval was given by Institutional ethical committee.

The research protocol was in accordance with the Helsinki Declaration of studying human subjects.

Study Subjects

Study population consisted of 100 subjects. 50 subjects were recruited as cases known to have Metabolic Syndrome as per IDF (International Diabetic Federation) guidelines for the study. Met S cases were further divided into two groups based on presence and absence of SCH. 50 age and sex matched healthy individuals were also recruited as controls.

Inclusion criteria

The inclusion criteria for enrolment into the study included the IDF definition of Met S which includes: Central Obesity (Waist circumference: ≥ 90 cm for Males and ≥ 80 cm for Females). Plus any two or more of the following 1) Hypertriglyceridemia-Fasting Triglycerides ≥ 150 mg/dl or specific medication, 2) Low HDL cholesterol: ≤ 40 mg/dl (Males), and ≤ 50 mg/dl (Females), or specific medication, 3) Hypertension: Blood pressure ≥ 130 mmHg Systolic or ≥ 85 mmHg Diastolic or previous diagnosis or specific medication and, 4) Fasting plasma glucose ≥ 100 mg/dl or specific medication or previously diagnosed type 2 diabetes.

Exclusion criteria

Exclusion criteria included a history of any acute and chronic infectious disease, liver disorders, renal disorders, congestive cardiac failure, pregnant women and patients on medications which may have affected study parameters.

Informed written consent was obtained from study population. Necessary clinical and physical examination of the study population was done especially pertaining to Met S.

Sample Collection

Venous blood samples were collected from the study subjects in a plain vial under sterile conditions. Routine investigations were done on the same day. The serum for adiponectin, insulin, thyroid hormone - T₃, T₄ and TSH were then stored at -20°C till serum was batch analyzed for different parameters.

Routine Laboratory Investigation

Routine investigation was done by using spectrophotometry method on Beckman Coulter CX-4 and CX-9 Random Access Autoanalyzer and reagents used of Centronic GmbH Reagents, In-vitro-diagnostics (Germany), Randox Laboratories (United Kingdom), Beckman Coulter – Clinical Diagnostic (California, USA), and Merck & Co (Germany).

Special Investigation- insulin resistance and adiponectin

Serum Insulin, TSH, T₃, and T₄ hormone were analysed by chemiluminiscence based immunoassay (CLIA) on Beckman Coulter Access 2 Immuno-assay system by using closed system Beckman reagent kit, California, USA. Normal reference value of T₄ = 2.5–3.9 pg/ml, T₃ = 0.6–1.12 ng/dl and TSH = 0.34–5.6 μIU/ml was considered [15].

Insulin resistance was studied by measuring fasting blood sugar, post prandial blood sugar, glycated haemoglobin (HbA₁c), serum insulin and HOMA-IR. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was adopted for assessing β-cell function and insulin resistance from fasting blood glucose and serum insulin levels [16].

HOMA-IR was used to calculate by formula which is follows as:

\[
\text{HOMA-IR} = \frac{\text{Serum glucose (mg/dl)}}{405} \times \frac{\text{Plasma Insulin (μU/ml)}}{
\]

Serum adiponectin levels were measured by sandwich ELISA method using the commercially available, human adiponectin Enzyme Immuno Assay kit (EIA-4574) by DRG International Inc.(USA).

Quality check of all the routine and immunoassays were routinely performed by participation in internal quality-control programs provided by Randox (Northern Ireland, United Kingdom) and Bio-Rad (Hercules, CA, USA). Our lab was also participating in the external quality control program namely "RIQAS International Quality Assessment Scheme/Proficiency testing" provided by Randox (Northern Ireland, United Kingdom).

STATISTICAL ANALYSIS

Variables measured were expressed as mean ± standard deviation. Comparisons of mean between two groups were performed using independent-sample t tests (Student’s t test). All statistical analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA), and a p value less than 0.05 was considered statistically significant. Pearson’s correlation was used to analyse the association among various study parameters. ROC curve was used to detect threshold level of study variable to detect SCH in Met S patient with
maximum sensitivity and specificity. Binary Logistic Regression analysis was used to analyse the predictability of Met S by study variables.

**RESULTS**

In the study 100 subjects were recruited into two groups: 50 patients of Met S and 50 age and sex matched healthy controls. In Met S group, 22 subjects had subclinical hypothyroidism (SCH) and 3 subjects had overt hypothyroidism, while rest of the individuals were euthyroid. In healthy control group, 4 subjects had subclinical hypothyroidism, while rest of the individuals were euthyroid. Demographic profile of study subjects is shown in Table 1. Met S study group had obesity and associated features such as deranged glucose and lipid Profile as compared to healthy controls.

**Thyroid function status in Study Population**

As shown in Table 2, mean T3 and T4 levels were not statistically different in two groups. While mean TSH levels were significantly higher in Met S cases compared to controls (5.7 ± 1.2 μIU/ml vs. 2.3 ± 1.6 μIU/ml, P < 0.001).

**Serum Adiponectin levels in Study Population**

As shown in Table 2, the mean serum adiponectin levels were significantly lower in cases (10 ± 2 μg/ml) as compared to controls (18.2 ± 7.2 μg/ml, P < 0.001). This states that Met S cases had insulin resistance as indicated by lower levels of serum adiponectin as compared to healthy control.

Moreover on ANOVA analysis, the mean serum adiponectin levels were lower in thyroid dysfunction Met S cases (Sub Clinical Hypothyroidism and Overt Hypothyroidism) as compared to Euthyroid Met S cases as shown in Table 3 although it was not statistically significant.

**Correlation Analysis of Adiponectin**

On Pearson’s correlation, serum adiponectin showed positive correlation with HDL and negative correlation with BMI, Systolic and Diastolic BP, Fasting Blood Glucose, Post Prandial Blood Glucose, HbA1C, Serum Insulin, HOMA-IR, Serum Triglycerides and TSH although FBS, HbA1C, HOMA-IR and TSH showed statistically significant correlation as shown in Table 4. Fig. 1 shows the scatter diagram of correlation between serum TSH and serum adiponectin. Fig. 2 shows the
Table 4 Correlation of TSH and Adiponectin with study variables

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TSH r value</th>
<th>p value</th>
<th>Adiponectin r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.240</td>
<td>0.016*</td>
<td>-0.161</td>
<td>0.110</td>
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<tr>
<td>Systolic BP</td>
<td>0.252</td>
<td>0.011*</td>
<td>-0.145</td>
<td>0.150</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.231</td>
<td>0.020*</td>
<td>-0.226</td>
<td>0.024</td>
</tr>
<tr>
<td>FBG</td>
<td>0.433</td>
<td>&lt; 0.001*</td>
<td>-0.371</td>
<td>0.000*</td>
</tr>
<tr>
<td>PPBS</td>
<td>0.190</td>
<td>0.058</td>
<td>-0.177</td>
<td>0.077</td>
</tr>
<tr>
<td>HbaA1C</td>
<td>0.463</td>
<td>&lt; 0.001*</td>
<td>-0.382</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.287</td>
<td>0.004*</td>
<td>-0.183</td>
<td>0.068</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.520**</td>
<td>&lt; 0.001*</td>
<td>-0.303</td>
<td>0.002**</td>
</tr>
<tr>
<td>Triglyceride (TG)</td>
<td>0.219</td>
<td>0.280</td>
<td>-0.130</td>
<td>0.198</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>-0.59</td>
<td>&lt; 0.001*</td>
<td>0.150</td>
<td>0.136</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.440</td>
<td>&lt; 0.001*</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>TSH</td>
<td>1</td>
<td>-</td>
<td>-0.440</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

*p value ≤ 0.05 is considered statistically significant. r = Pearson's correlation coefficient
**= Spearman's correlation coefficient

Fig. 1 Correlation analysis of TSH with serum adiponectin in Met S group

Binary Logistic Regression Analysis

A logistic regression analysis was conducted to predict the development of Met S by using serum adiponectin and TSH as a predictor. The logistic regression model was statistically significant, $\chi^2(2) = 106$, $p < 0.001$. The model explained 87.3% of the variance in Met S. Nagelkerke’s $R^2$ of 0.87 indicating a moderately strong relationship between prediction and grouping. We found that TSH ($p < 0.001$, Odd ratio=7.5, 95% CI = 2.89–19) and adiponectin ($p < 0.001$, Odd ratio = 0.64, 95% CI=0.49–0.83) had significant predictability for Met S. Exp(B) value indicates that when serum TSH is raised by one unit the odds ratio is 7.5 times as large and therefore individuals are 7.5 times more likely to develop Met S.
times more likely to develop Met S. Likewise when
serum adiponectin is raised by one unit the odds ratio
is 0.64 times as small and therefore individuals are 0.64
times less likely to develop Met S.

**DISCUSSION**

In the present study, Met S cases were found to have
insulin resistance and underlying thyroid dysfunction
in the form of SCH. Mean serum adiponectin levels
were also found to be lower in Met S cases as com-
pared to controls; moreover the serum adiponectin lev-
els were lower in Met S cases having SCH as compared
to Met S cases without thyroid dysfunction. Hence, a
positive association between decreased serum adiponec-
tin levels with insulin resistance in Met S patients in
the presence of subclinical hypothyroidism was found.
Subclinical hypothyroidism in Met S may cause altered
adipocyte physiology which causes decreased release
of insulin sensitising adipokines such as adiponectin
which may lead to insulin resistance and associated co-
morbidities such as future development type II DM.

Adipose tissue is now considered as an active endo-
crine organ that produces various adipokines such as
TNF-α, IL-6, adiponectin etc which plays an import-
ant role in modulating insulin sensitivity. Alterations in
the levels of adipokines may lead to insulin resistance

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Fig. 2 Correlation analysis of TSH with HOMA-IR in Met S group

Fig. 3 ROC curve for Serum TSH

Fig. 4 ROC curve for Serum adiponectin
in various insulin sensitive tissues such as skeletal muscle and adipose tissue [18]. The cross talk between adipose tissue and other biological systems is through the adipokines. There are various adipokines especially adiponectin, which has received growing attention among researchers due to its direct vascular and metabolic effects [19]. It has been seen that plasma adiponectin levels decreases in obesity related to morbidities such as diabetes mellitus, Met S and coronary artery disease. Adiponectin play an important role in the insulin sensitivity in normal healthy individuals [20].

**Insulin sensitizing action of adiponectin: Probable mechanism**

Adiponectin is a 30 kDa protein which is composed of 244 amino acids with C-terminal globular domain and a collagen-like N-terminal domain. It is a product of the APM1 gene, and is mainly secreted by adipocytes. Various studies have shown that adipocytes with exhausted lipid storage, filled with fatty acids inhibit transcription of the adiponectin gene by secreting various pro-inflammatory, prothrombotic and angiogenic factors [21]. The adiponectin performs its biological function by adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) [22]. It increases the uptake of glucose and fatty acid oxidation in muscle by AdipoR1 and decreases glucose production by the liver by AdipoR2 [23].

In an experimental study it has been seen that adipocytes overexpressing adiponectin causes accelerated adipocytes differentiation and insulin-stimulated glucose transport activity. Hence adiponectin play a key role in adipocytes differentiation and insulin sensitivity. It also inhibits the secretion of various pro-inflammatory mediators such as IL-6, IL-8, MIP (Macrophage Inflammatory Protein), MCP-1 (Monocyte Chemotactic Protein-1), which leads to inhibition of storage of lipids and insulin sensitivity in adipocytes. Adiponectin also increases insulin-stimulated tyrosine phosphorylation of insulin receptors which lead to insulin sensitivity [23]. Individuals with obesity, insulin resistance and diabetes are usually presented with decreased circulating levels of adiponectin [23].

In the present study, mean serum adiponectin was found to be lower in cases of Met S as compared to healthy controls; the difference was found to be significant. Also, cases of Met S with SCH were found to have lower level of adiponectin compared to Met S cases without SCH however the difference was not found to be significant owing to small sample size. However strong association was found on the likelihood of low levels of adiponectin with Met S cases.

**Role of thyroid hormone in insulin resistance**

Thyroid hormones play an important role in body energy homeostasis and metabolism of carbohydrate, protein and lipids. Thyroid hormone action is peculiar in terms of insulin as in one way it opposes the action of insulin by stimulating the hepatic gluconeogenesis and glycolgenolysis and in other way it acts synergistically with insulin by facilitating glucose disposal and utilization in peripheral tissues by up-regulating the expression of genes such as glucose transporter type-4 (GLUT-4) and phosphoglycerate kinase which are involved in glucose transport and glycolysis, respectively [24].

It has been reported in various studies that thyroid abnormalities in the form of sub clinical hypothyroidism is prevalent in Met S. In our study, we found 44 % and 6 % cases of Met S having sub clinical and overt hypothyroidism respectively. A study conducted by Gyawali P et al. on the prevalence of sub clinical hypothyroidism in Met S cases of Nepal found that SCH was present in 29.92 % cases of Met S [25]. In various other studies, the prevalence of subclinical hypothyroidism has been estimated to be from 1.1 % to 8 %. The reason for wide discrepancies in prevalence of SCH in Met S may be related to variances in the ethnic group analyzed, intake of iodine, population age, sample size of study population etc [26].

The thyroid dysfunction is prevalent in obesity and its associated co morbidities such as Met S as compare to healthy individuals. In the presence of SCH, the physiology of adipocytes may alter which can contribute to other metabolic disorders such as Met S [14]. It has been seen that adipocytes express high levels of TSH receptors, which points towards participation of TSH hormone in modulating adipocyte physiology such as altering adipokine secretion [24].

Chen Y et al. studied insulin resistance, lipids and adipokine levels in 427 patients of thyroid dysfunction and 355 healthy controls. They found altered lipid and adipokine profile along with insulin resistance in thyroid dysfunction patients as compare to healthy individuals. Although they concluded that adipokines may serve as a link between thyroid dysfunction and insulin resistance but they could not find any significant difference in adipokine levels in hypothyroidism cases and control [12]. Gierach M et al. studied insulin resistance in sub clinical hypothyroidism and overt hypothyroidism and found that there is an impairment in the translocation of GLUT-4 in plasma membrane of monocytes which also hints toward the importance of thyroid hormone in modulating insulin sensitivity [27].

Present study provides evidence regarding the decreased serum adiponectin in Met S patient with thyroid dysfunction in the form of SCH. Thyroid hormone profile in SCH shows an increase in TSH level while T_3 and T_4 levels are maintained. Likelihood of high serum TSH levels and low serum adiponectin with Met S was found to be significant on logistic regression analysis.

Increased TSH levels binds to TSH receptor on adipocytes and brings about altered thyroid hormone signalling in adipocytes, leading to decreased release of insulin sensitising mediator – adiponectin which ultimately lead to insulin resistance in Met S cases. Although the exact biochemical mechanism of the same is still unknown, however the hypothesis holds good as it has been shown that TSH receptor is over expressed on adipocytes in Met S [28]. Also, levothyroxine replacement therapy in SCH, showed reversal of metabolic abnormalities in Met S patients [29]. Disturbances in pituitary thyroid axis have also shown to alter serum adipokine levels such as adiponectin [30]. Future research in this aspect may show this finding as
Ahirwar AK et al. /TSH, Adiponectin and Insulin Resistance in Metabolic Syndrome

Subclinical Hypothyroidism in Metabolic Syndrome

[Diagram]

- Increase in serum TSH
- Altered adipocytes homeostasis
- Dysfunctional Adipocytes
- Decrease secretion of insulin sensitising Adipokines - Adiponectin
- causes Insulin Resistance
- Increase Risk of type 2 Diabetes Mellitus & Cardiovascular disease

Fig. 5 Probable link between the insulin resistance and thyroid dysfunction in Metabolic Syndrome according to our study.

...a vicious circle.

Strength of our study is well characterised study population and standardized techniques to measures various parameters of the study. Limitation of our study was that it had small sample size and study populations were Indian ethnic group so it may not be generalised to other ethnic groups. Though, we found a strong association between SCH and insulin resistance in Met S, but just association would be inadequate to establish the cause and effects relationship. A prospective study design to look for development of insulin resistance and DM II in cases of Met S with SCH would be more appropriate study design. Also molecular mechanism behind it needs to be explored. We measured the total adiponectin level while high molecular weight adiponectin is the active form and thus could be more informative.

CONCLUSION

Thus, it could be concluded that under the influence of SCH in Met S, adipocytes behave abnormally and causes decreased release of insulin sensitive adiponectin which may lead to insulin resistance and associated co-morbidities as shown in Fig. 5 [31].

Since SCH remain undiagnosed for long time in Met S, this may cause insulin resistance in future and associated metabolic abnormalities such as diabetes mellitus and cardiovascular disease. Hence all Met S cases should be screened and followed up for any future development of SCH and be appropriately managed by thyroxin replacement therapy. Based upon ROC analysis, a higher cut-off of 5.64 IU/L for TSH and a lower cut-off of 10.50 μg/ml for adiponectin for development of insulin resistance in Met S patients with SCH have been recommended. Even the elevated serum TSH levels could be taken as an early biomarker for developing Met S [31, 32]. The same should be validated on a larger sample size.

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