Altered Fructosamine and Lipid Fractions in Subclinical Hypothyroidism

ABSTRACT

Background: Thyroid function disorders lead to changes in the lipoprotein metabolism.

Objectives: To study the lipid and the glycaemic abnormalities in subclinical hypothyroidism cases and to compare the same with the euthyroid, overt hypothyroid and the hyperthyroid subjects.

Methodology: Four groups, euthyroid (Group-I), hypothyroid (Group-II), subclinical hypothyroid (Group-III) and hyperthyroid (Group-IV), which consisted of 30 subjects each, of either sex, were aged 25-55 years, underwent Fasting Plasma Glucose (FPG), fructosamine, lipid profile and T3, T4 and TSH estimations. The subjects who were on lipid lowering or thyroid disorder drugs and known diabetics were excluded from the study.

Results: In Group-III, all the lipid fractions were comparable to those of Group-II and they were significantly deranged, as compared to those of Group-I. The fructosamine levels were significantly higher in Group-II and Group-III (p<0.05), but the subclinical hypothyroid pool had statistically lower levels than the hypothyroid pool (376.63±54.73, 587.80±65.10). In the Group-IV patients, the LDL-C levels were significantly higher as compared to those in the euthyroid pool. The fructosamine levels were significantly lower in comparison with both the euthyroid and the hypothyroid pools (both in Groups-II and III). The FPG levels were higher in all the classes of the thyroid abnormalities (subclinical hypothyroid not significant) but within the reference range of 70-100mg/dl.

Conclusion: Since the lipid derangement in subclinical hypothyroidism is on par with that in overt hypothyroidism, the subclinical hypothyroid cases also need to be treated similarly. The fructosamine values which are largely in excess of the FPG values, indicate a higher propensity to glycation and a decreased turnover of the proteins in the hypothyroid and the subclinical hypothyroid pools. Vice versa is true of the hyperthyroid pool. Fructosamine can be included in the thyroid work up of the patients to assess the metabolic function and the subsequent response after the initiation of the therapy.

Key Words: Fructosamine, Lipid Profile, Hyperthyroid, Hypothyroid, Subclinical Hypothyroid

INTRODUCTION

The thyroid hormone is known to play a role in the regulation of the synthesis and in the metabolism and the mobilization of lipids. Subclinical Hypothyroidism (SCH) is a clinical condition which is characterized by elevated serum Thyroid Stimulating Hormone (TSH) concentrations and with normal serum levels of the thyroid hormones i.e T3, T4, freeT3 and freeT4. By affecting the metabolism of the lipids, hypothyroidism accelerates the process of atherogenesis and it increases the cardiovascular risk. Hyperthyroidism exhibits an enhanced excretion of cholesterol and an increased turnover of LDL-cholesterol, resulting in a decrease in the total cholesterol and LDL-cholesterol levels and in raised HDL-cholesterol levels [1]. Fructosamine (glycated albumin) is generally used for assessing the glycaemic control in diabetics over a 2-3 week period. When the fructosamine values were compared between the hypo and the hyperthyroid patients, it was found that the fructosamine levels and the fructosamine per albumin ratio were significantly lower in the patients with Grave’s disease than in the normal subjects, while they were significantly higher in the patients with primary hypothyroidism [2,3]. The studies which have compared the fructosamine and the lipid fractions in SCH are few and hence, the present study was undertaken to observe the said changes in SCH and to compare them with those in the overt hypo, hyper and the euthyroid controls.

METHODOLOGY

With the approval of the institutional ethics committee of Kasturba Medical College, Mangalore, Karnataka, India and the informed consent of the participants, a total number of 120 participants of ages 25 to 55 years were chosen and they were divided into 4 groups of 30 patients each. i) Euthyroid, normal lipid status individuals, ii) untreated hypothyroid cases iii) Newly diagnosed SCH cases and iv) Hyperthyroid cases before the initiation of the therapy. The fasting lipid profile [Total Cholesterol (TC), Triglycerides (TG), HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol, TC/HDL and LDL/HDL ratios], the thyroid profile (total T3, T4 and TSH) and the glycaemic profile which consisted of fasting plasma glucose and serum fructosamine were estimated/calculated in all the groups. The diagnoses of hypo and hyperthyroidism were established, based on the clinical signs and symptoms and the T3, T4 and TSH estimations. Known diabetics or the patients who were on treatment with lipid lowering drugs or thyroid drugs were not included in the study. The estimation of the thyroid profile was done by the Lilac kit by using a chemiluminescence method. The serum lipid profile was estimated by the enzymatic CHOD-P0D method for TC, by the GPO-PAP method for TG and by the CHOD-P0D/ Phosphotungstate method for HDL-cholesterol. These estimations were carried out by using an Erba- Chem Pro-5 semi automated analyzer. LDL-Cholesterol and VLDL-Ch...
Lesterol were calculated by Friedwald’s formula and the TC/HDL and the LDL/HDL ratios were noted. FPG was estimated by the GOD-POD method and serum fructosamine was estimated by the NBT reduction method [4], where, under alkaline conditions, fructosamine rearranges to the eneaminol form, which reduces NBT to formazan. The absorbance at 530 nm is measured at 2 time points and the absorbance change is proportional to the fructosamine concentration.

STATISTICAL ANALYSIS

The Kruskal Wallis test, the Mann Whitney-U test and the Fisher test were applied for the descriptive analysis and the correlation was done by the Pearson’s correlation coefficient.

RESULTS

The mean ages of the Group-II, Group-III and the Group-IV patients were 40.53±9.73, 41.50±10.24 and 41.17±8.64 respectively.

[Table/Fig-1] shows the serum TSH and the total thyroid hormone levels of the 4 groups. As compared to the controls, the mean TSH level was significantly higher with lower T3 and T4 values in the Group-II patients and vice versa in the Group-IV patients. Normal T3 and T4 values and raised TSH levels (upto 20 μIU/ml) were considered to be suggestive of SCH (Group-III).

[Table/Fig-2] illustrates the thyroid status of the study groups. FPG and fructosamine were compared in the hypothyroid pool for increased TC, TG, LDL-Cholesterol and VLDL-Cholesterol levels and TC/HDL and LDL/HDL ratios and decreased HDL-Cholesterol levels. The lipid fractions in the SCH cases were comparable to those in the overt hypothyroid group. Higher LDL-Cholesterol and LDL/HDL-Cholesterol ratios were observed in the hypothyroid group.

[Table/Fig-3] has compared the FPG and the serum fructosamine concentrations of the study groups. FPG and fructosamine were significantly elevated in the Group-II and Group-III patients. The fructosamine levels were significantly lower in the Group-IV patients, even with significantly elevated FPG levels.

DISCUSSION

The hypothyroid pool: A lack of thyroid hormones in hypothyroidism causes an elevation of the cholesterol synthesis due to an increase in the cholesterol synthesis and absorption, a decrease in the hepatic lipase and the lipoprotein lipase activities, defects in the receptor-mediated catabolism of LDL-cholesterol [1], an increase in the oxidation of plasma cholesterol, mainly TC and LDL-cholesterol and a decrease in the HDL receptors on the hepatocytes.

In the hypothyroid pool which was diagnosed, based on the raised TSH and the lower T3 and T4 levels, the triglycerides were found to be significantly elevated, along with TC, LDL-Cholesterol and the TC/HDL and the LDL/HDL ratios and there was a significant decrease in serum HDL-Cholesterol level [Table/Fig-2]. Evidence is available, to say that not only TC and LDL-cholesterol, but that the triglycerides were also independent risk factors for Coronary Heart Disease (CHD), cerebro vascular disease, and peripheral artery disease [5,6]. Furthering the importance of the triglycerides in this study, a significant, negative dependent correlation of only TG and VLDL-cholesterol (which is a function of TG), with T3 was found in this group [TG - r = -0.483, p = 0.007] (VLDL-cholesterol - r = -0.473, p = 0.008). Of notable importance was a marked increase in the LDL cholesterol levels and consequently, the LDL/HDL ratio. The findings in relation to the lipid status point to the high susceptibility of the hypothyroid subjects to the development of cardiovascular diseases.

Fructosamine glycated albumin is best known for its use as a tool for assessing the glycaemic status, particularly in diabetics, where the glucose levels in plasma are high. All the hypothyroid cases had normal FPG values (88.63 ±10.33 mg/dl) [Table/Fig-3] as per the reference range (70-100 mg/dl), but the mean value was significantly higher as compared to that in the normal euthyroid controls (p = 0.020). Despite the normoglycaemia of the hypothyroid patients, fructosamine was greatly increased in them, which could be due to the decreased turnover of the plasma proteins in hypothyroidism. Another approach to the explanation could be, the raised oxidant milieu in hypothyroidism [7,8]. In hypothyroidism, the associated oxidative stress is the consequence of both the increased production of free radicals and the reduced capacity of the antioxidant defence. The enzyme, thryroperoxidase oxidizes iodide in the presence of H2O2 by using NADPH as a coenzyme. When the thyroid gland functions subnormally and when not enough iodination of the thyroid hormones takes place, the thyroid gland becomes a major site of a dangerous H2O2 generation. The cascade gets activated, resulting in raised TSH levels, thus increasing the production of H2O2, depleting the defense mechanisms
like glutathione peroxidase, reducing the synthesis of T3 and T4, further increasing the TSH levels and worsening the functioning of the thyroid gland [9]. Christ-Crain et al., [10], showed elevated C Reactive Protein (CRP) and homocysteine levels with progressive thyroid failure and postulated these as additional risk factors for the cardiovascular risk in hypothyroidism. Inflammation plays an important role in the progression and the complications of atherosclerosis. An inflammatory state, as is seen in hypothyroidism, adds to the generation of free radicals and the resultant ill effects of the same. When Segadeet al., [11], and Fujita et al., [12], studied the effects of different serum proteins on the fructosamine concentration, they found a significant correlation, not only with albumin, but also with the β and γ globulins, IgG, IgA, IgM and the total protein concentration. Any inflammatory process triggers the γ – globulin synthesis. The generation of free radicals in the inflammatory state, combined with the raised γ – globulins and the propensity of the γ – globulins to glycation, could be the basis of the increased glycation of the proteins in hypothyroidism. Hypothyroidism is also associated with hyperinsulinaemia [13]. The slowed metabolic state of hypothyroidism not only slows the rate of glucose absorption from the gastro intestinal tract, but it also reduces the glucose utilization because of the insulin resistance [13,14]. In the current study, the significantly higher FPG of this group in comparison with that of the reference group, pointed to the possibility that hyperinsulinaemia and insulin resistance may be causing small excursions in the glucose levels in the prandial and the post prandial states. This needs to be ascertained by taking up future studies, by using the Glucose Tolerance Test (GTT) or reference meals. A significant correlation of FPG with fructosamine was found in this group (r = 0.916, p = 0.000). The glycated proteins which are thus formed, themselves are sources of free radicals, further aggravating the free radical damage. Also, they get oxidized to Advanced Glycation End products (AGE) [15], which can be persistent and cumulative in the tissues. Fructosamines may undergo oxidative degeneration via the enolic intermediates. The non oxidative degradation of fructosamine involves the reversal of the Amadori reaction to form the Schiff’s base, which after hydration, forms free amine and glucose. The degradation of fructosamine gives rise to superoxide radicals which contribute to the oxidative stress [16].

Thus the postulations put forward for the abnormally high fructosamine levels in the absence of clinical hyperglycemia in the hypothyroid patients are:

1. Decreased metabolism leading to decreased turn over of proteins and thus prolonging their half - life.
2. Increased oxidative stress causing increased glycation of proteins.
3. Low grade inflammation adding to the free radical formation and its effects. Raised immunoglobulins in response to inflammation and preferential glycation rates of immunoglobulins.
4. Altered glucose homeostasis with decreased absorption and conversely decreased utilization also associated with hyper insulinaemia and insulin resistance probably causing transient elevations in the glucose concentrations thus contributing to glycation of serum proteins.
5. The tendency of glycated proteins to accumulate in tissues resisting easy proteolysis and being further source of free radicals.

The subclinical hypothyroid pool: The lipid derangements which were observed in the overt hypothyroid pool were replicated in the SCH pool also, to a similar extent [Table/Fig-2]. The fructosamine levels were not as high as those in the hypothyroid group. The above findings were recorded inspite of choosing a low cut off value of 5.6-20 μIU/ml for TSH for the SCH patients. Also, the mean age of the patients was much lower (41.50±10.24) than the older age (> 60) which was reported in other studies [17,18]. In view of the cardiovascular risk which was involved, the SCH patients thus need to be cautiously monitored and if the clinical features suggest, they should be treated as overt hypothyroid cases.

The hyperthyroid pool: There exists a state of hypermetabolism in hyperthyroidism, resulting in increased muscle protein breakdown and enhanced metabolic activities [19,20]. A reversal of the findings in hypothyroidism can be expected in the hyperthyroid pool. Surprisingly, the LDL-cholesterol concentration and the LDL/HDL ratio remained elevated as compared to those in the euthyroid controls. The TC and the TG values were also slightly higher, but they were not statistically significant. The HDL-cholesterol levels did not differ in the hyperthyroid and the euthyroid pools.

Most of the studies that have compared the cholesterol levels in hypo and hyperthyroidism [21,22], have reported significantly lower LDL-cholesterol levels in hyperthyroidism as compared to those in the hypothyroid pool (raised LDL-cholesterol). The thyroid hormone (T3) is known to affect the LDL-cholesterol levels. The promoter of the LDL receptor gene contains a thyroid hormone responsive element (TRE) and T3 modulates the gene expression of the LDL-receptor [23]. Accordingly, lower levels of LDL-cholesterol can be expected with raised T3 levels. Diekman et al., [22], studied the polymorphism in the LDL-receptor and reported that the main determinant in the changes in the LDL-cholesterol concentration, is the change in the plasma free T4 and not polymorphisms of the LDL-cholesterol receptor. In human plasma, the thyroid hormones are transported primarily by the T4 binding pre-albumin and the serum albumin. A small fraction of T4 is bound to the plasma lipoproteins (0.8% to VLDL-cholesterol, 6.7% to LDL-cholesterol and 92% to HDL-cholesterol) [24,25]. T3 binds to the same proteins, but with lesser affinity. The T4 – LDL complex is recognized by the LDL receptor and this interaction provides an additional mode of the T4 entry into the cell [26]. As the T3 and T4 levels are in excess in hyperthyroidism, it may be expected that this excess entry of the hormones into the cells exerts its effects as the signs and symptoms of hyperthyroidism. To carry the extra load of T4, the LDL-cholesterol levels may be increased. In support of this statement, a correlational analysis revealed a significant positive association of T3 with LDL-cholesterol (r = 0.389, p = 0.034) and a weak significant negative association of T3 was found with HDL-cholesterol (r = -0.539, p = 0.052). Sundaram et al., [21] and Oge et al., [27] studied the LDL-cholesterol oxidation in hypo and hyperthyroidism and found that in both the cases, the LDL-cholesterol oxidation was increased as compared to that in the euthyroid cases. They attributed this to the increased generation of free radicals that accompanied the lipid peroxides in hyperthyroidism. Hyperthyroidism is not usually associated with atherosclerosis. The cardiac complications are usually arrhythmia or congestive heart failure, which are secondary to the hypermetabolic state [28]. The aspect of the LDL-cholesterol oxidation in hyperthyroidism and the role of the enhanced LDL-cholesterol oxidation in the cardiac disease process in these patients requires further debate.
viciously reported data on carbohydrate metabolism and the fructoseamine levels in hyperthyroidism [2,29,30]. A significant positive association was found between FPG and fructoseamine (r = 0.977, p < 0.001). There exists a state of oxidative stress even in hyperthyroidism [31], which should have raised the possibility of the proteins getting glycated, but the protein turnover must be largely in excess of the probabilities of their glycation. Ford et al., [29] and Lee et al., [30] showed an increase in Hba1c which corresponded to the raised glucose concentrations, but also to a decrease in the fructoseamine levels and the albumin concentration. These conflicting findings and results, where one class of proteins resisted glycation and the other corresponded to the plasma glucose concentrations, need to be explored.

**CONCLUSION**

With respect to the lipid derangements, no difference was found between the hypothyroid and the SCH pools, thus putting the patients with SCH also at an equal risk of CHD. Views differ on the treatment options for SCH. While there are schools of thought which would like to defer the treatment of the SCH cases until they exhibit clinical hypothyroidism, the dyslipidaemia which is observed and its consequent effects prompt an initiation of the thyroxine replacement therapy in the SCH cases also. The disproportionate increase in the fructoseamine levels as compared to that in the FPG levels point to the possibility of a much complex carbohydrate metabolism and the glycation of proteins. It has been hypothesized that the elevated fructoseamine levels could be due to a decreased protein turnover, which thus prolongs the half-life of the proteins, the increased glycation of proteins due to increased oxidative stress, inflammation, an altered glucose homeostasis with hyperinsulinaemia and insulin resistance and an increased accumulation of the glycated proteins in the tissues, which resist proteolysis. Fructoseamine can be included in the work up. In hyperthyroidism, the protein turnover must be largely in excess of the evidence of oxidative stress and the altered glucose homeostasis, which explains the lowered fructoseamine levels in this group. The higher levels of FPG in all the groups of thyroid abnormalities (SCH-not significant) and a significant positive correlation with fructoseamine among all the groups, further support the altered carbohydrate metabolism in them. Detailed studies need to be taken up to understand the complex interaction of the glucose homeostasis, the protein turnover and the glycation of proteins in the thyroid diseases.

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