Effect Of Levothyroxine Therapy On Reduced Glutathione And High-Sensitivity C - Reactive Protein Among Patients With Hypothyroidism

Nidhi Gohil¹, Anita Sinha², Vipul Navadiya³, Aashal Shah*4

¹Drug Safety Physician, Springer nature technology and publishing solutions, Pune, Maharashtra
²Associate Professor, Pandit Dindayal Upadhyay Medical College, Sardar Civil Hospital Campus, Rajkot, Gujarat - 360001
³Tutor, Government Medical College and New Civil Hospital, Majuragate, Surat, Gujarat – 395001.
⁴Tutor, GMERS Medical College and Civil Hospital, Valsad, Gujarat - 396001

ARTICLE INFO

Objectives – Hypothyroidism has been associated with an increase in the oxidative stress and cardiovascular (CV) morbidity. This study evaluates the effect of 12-weeks levothyroxine replacement therapy on reduced glutathione (GSH) and high-sensitivity C-reactive protein (hsCRP) levels. It also assesses the association between hsCRP levels and CV risk.

Methods – Prospective, observational study carried out at a tertiary care hospital for one year. Forty newly diagnosed adult hypothyroid patients started on levothyroxine treatment were included in the study. The patient’s demographic details, relevant history and levothyroxine treatment details were recorded in patient data sheet. Follow up was done after 12 weeks of continuous levothyroxine therapy. Two blood samples were collected from all participants for comparison of pre- and post-treatment GSH and hsCRP levels. hsCRP level was used to grade the risk of cardiovascular disease (CVD). Results were analysed using paired t-test. P value < 0.05 was considered statistically significant.

Results - Among the 40 enrolled patients, 85% were females and the mean age was 40.76 ± 11.05 years. There was a significant increase in thyroid hormones and significant decrease in thyroid stimulating hormone after 12 weeks of levothyroxine therapy. A significant increase in the hsCRP levels was also noted. There was an increase in the number of patients in high CVD risk group from 17 to 29. GSH levels were also increased, but it was statistically insignificant.

Conclusion - Increase in GSH and hsCRP levels after levothyroxine treatment suggesting that it may not protect patients from cardiovascular morbidity and mortality.

Keywords: Atherosclerosis, Cardiovascular risk, Inflammation, Oxidative stress

Corresponding Author: Aashal Shah, Tutor, GMERS Medical College and Civil Hospital, Valsad, Gujarat - 396001

Br J Phar Med Res Copyright©2021 Nidhi Gohil et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license.
INTRODUCTION:
Hypothyroidism is defined as an overt or subclinical deficiency of thyroid hormones, which can be either complete or partial. Hypothyroidism is among the most common endocrine disorders in India. According to the nationwide surveys, India’s burden of thyroid diseases has increased up to 42 million. The approximate hypothyroidism prevalence in India is 11%. The prevalence of subclinical and clinical hypothyroidism in the developed countries is about 4-15% and 4-5%, respectively. The spectrum of hypothyroidism ranges from an asymptomatic condition to an overt state of myxoedema and multisystem failure. Subclinical hypothyroidism (SCH) is defined by high levels of serum thyrotropin (TSH) along with normal free thyroxine (FT4). On the other hand, in Overt Hypothyroidism (OH) TSH is elevated and FT4 is below normal. Thyroid replacement therapy using the synthetic hormone levothyroxine is the gold standard of treatment for hypothyroidism. The dose of levothyroxine is usually started low and gradually increased on the basis of serum TSH levels to achieve adequate level of control.

Thyroid hormones are responsible for homeostatic regulation of redox-balance. Thyroid hormones regulate rate of metabolism in our body. Imbalance between the rate of generation of reactive oxygen species (ROS) and antioxidant defence system leads to oxidative stress. It interferes with intracellular signal transduction as well as physiologic adaptation. Also, there is oxidative DNA damage and lipid peroxidation. All this leads to a change in the intracellular redox status leading to the activation of protein kinases, such as, protein kinase C, tyrosine kinase cascades leading to changed cellular functions. There is a direct link between metabolic effects of thyroid hormones and oxidative stress. Hypothyroidism can alter the function of mitochondrial respiratory chain leading to enhanced free radical production. Hypothyroidism is associated with increased free radical production as well as decreased antioxidative defence function leading to higher ROS production. Excess thyroid stimulating hormone (TSH) alters oxidative stress processes. Thus, thyroid hormones play a vital role in oxidant-antioxidant system modulation.

Glutathione is a tripeptide formed from three amino acids namely, cysteine, glutamic acid and glycine. It is a vital antioxidant which protects our body from the reducing agents. It is an essential part of xenobiotic metabolism where it performs conjugation reaction and detoxifies the substrate. Reduced glutathione (GSH) serves as a cofactor for both, antioxidant enzymes and deiodinases which convert thyroxine to triiodothyronine. Alterations in GSH activity have been reported in hypothyroidism.

C-reactive protein (CRP) is one of the most sensitive and objective marker of inflammation. It may act in the pathogenesis of atherosclerosis via several mechanisms and thus, is an important predictor of cardiovascular risk. As it has a wide reference range, many highly sensitive methods have been developed for measurement of CRP for screening purpose. Usually, a high-sensitivity CRP (hsCRP) test is done to predict the risk of developing coronary artery disease as it is more sensitive than the standard test. As previously established, hypothyroidism has been associated with increased cardiovascular morbidity. Obesity, hyperlipidemia and hypercoagulable state in hypothyroidism accounts for accelerated atherosclerosis. Thus, the values of serum hsCRP are significantly raised in individuals with hypothyroidism.

Many studies have been conducted to determine the association between oxidative stress, hsCRP and hypothyroidism. But, there is a dearth of research studies on how the thyroid replacement therapy would affect oxidative stress and levels of hsCRP in hypothyroid patients. Inconsistent results have been reported in previous studies about the impact of levothyroxine replacement therapy on parameters of oxidative stress. Also, there is no clear data on the effect of levothyroxine replacement on hsCRP levels in hypothyroid individuals. Therefore, this study has been carried out with an aim to investigate the levels of hsCRP and GSH in patients with hypothyroidism and to evaluate the consequences of levothyroxine replacement therapy on both the
parameters in the same patients. Also, association between hsCRP levels and cardiovascular risk has been assessed in these patients 12 weeks after commencing levothyroxine therapy.

MATERIALS & METHODS
This was a prospective, observational study with an informal experimental design, which was carried out for a duration of one year in the Department of Medicine at New Civil Hospital, which is a tertiary care teaching hospital affiliated with Government Medical College, Surat, Gujarat. The study was commenced after the approval of Institutional Ethics Committee.

The inclusion and exclusion criteria for the study participants were defined as follows:

Inclusion criteria:
- Newly diagnosed hypothyroid patients (Reference values - TSH: 0.35 to 5.5 μIU/ml, FT4: 0.8 to 1.71 ng/dl, FT3: 2.02 to 4.43 pg/dl).
- Patients willing to be treated with levothyroxine therapy upon diagnosis of hypothyroidism.
- Patients aged between 18 and 65 years.
- Patients willingly giving consent to be part of this study.

Exclusion criteria:
To limit the confounding errors influencing laboratory or clinical parameters being investigated, a set of exclusion criteria were defined. They included:
- Subjects receiving antioxidant vitamin supplements for the past 3 months.
- Pregnant and lactating women.
- Patients having habit of alcohol and smoking.
- Patients receiving radioactive iodine treatment.
- Patients with chronic inflammatory disease and/or taking medications for the same.
- Patient with neurological involvement.

Sample size estimation:
Based on previous study[18], prevalence of hypothyroidism among thyroid abnormality suspected patients was 9.27%. Sample size was calculated using OpenEpi software version 2.3 with the power of 90% and 95% confidence interval and sample size was 48. Due to cost and time constraints involved in the study, purposive sampling with a sample size of 40 patients was decided for this study.

Methodology:
Forty (n = 40) newly diagnosed and confirmed cases of hypothyroidism who were going to be treated with levothyroxine were enrolled in the study from the medicine department in a random fashion. Before enrolment, all the patients were properly informed in a way that they understood about the study and their consent was recorded on a pre-approved informed consent form. The diagnosis of hypothyroidism was made by the treating physician of the medicine department. The patients were given tablet levothyroxine once a day for 12 weeks and the doses were decided according to their thyroid function tests. Only the patients who gave assurance to comply with the treatment and follow up were enrolled. The patient’s demographic details, relevant medical history and specifications regarding levothyroxine treatment were recorded in a predesigned, pre-approved patient data sheet. Follow up visit was done after 12 weeks of continuous levothyroxine therapy. Pre- and post-treatment levels of GSH and hsCRP were compared in the participants. Two blood samples were collected from all participants during the study for analysis of GSH and hsCRP levels. First baseline sample was collected after the diagnosis of hypothyroidism and before the start of levothyroxine therapy and second sample was collected 12 weeks later with continued levothyroxine treatment. Also, the level of hsCRP was used to grade the risk of cardiovascular disease (CVD). Patients with hsCRP levels less than 1 mg/L were considered low risk, whereas patients with hsCRP levels more than 3 mg/L were considered to have high cardiovascular risk. Patients with hsCRP levels between 1-3 mg/L were regarded as moderate risk for CVD.

Blood sample collection:
For the diagnosis of hypothyroidism, after 12 hours fasting, blood samples were collected. The thyroid profile (FT3, FT4 and TSH) was measured by chemiluminescence immunoassay based test (Siemens healthcare diagnostics, USA). The reference values
considered were: TSH: 0.35 to 5.5 μIU/ml, FT4: 0.8 to 1.71 ng/dl, FT3: 2.02 to 4.43 pg/dl.

After diagnosis of hypothyroidism by thyroid profile test, 5 ml venous blood sample was collected in plain vacuette after taking informed consent for collection of blood for laboratory test. Cayman Glutathione assay kit procured from Labdhee laboratories was used to assess the GSH levels and High sensitive C-Reactive Protein latex-enhanced turbidimetric in-vitro immunoassay kit procured from AGAPPE Diagnostics LTD was used to measure hsCRP. Cayman’s GSH assay utilizes a recycling method, using glutathione reductase which is carefully optimized for GSH quantification. Specific anti-human CRP coated latex particles are used for hsCRP.

Data analysis:
Data was analysed using Microsoft excel 2013. The results were expressed as Mean ± Standard deviation. The results were analysed using paired t-test. P value < 0.05 was considered statistically significant.

RESULTS
In this study, 40 newly diagnosed & confirmed hypothyroid patients were enrolled. These 40 patients received levothyroxine therapy for treatment. Levels of GSH and hsCRP before starting levothyroxine therapy were considered as baseline and it was compared with the same parameters after 12 weeks of therapy. The mean age of the study population was 40.76 ± 11.05 years. Among the 40 enrolled patients, 85% (n = 34) were females and 15% (n = 06) were males.

Effect of levothyroxine treatment on thyroid profile
Table 1 gives the comparison of thyroid profile of the study population before levothyroxine treatment and 12 weeks after levothyroxine treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before treatment (n = 40)</th>
<th>levothyroxine treatment (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free T3 (pg/dl)</td>
<td>1.72 ± 0.683</td>
<td>2.96 ± 0.916*</td>
</tr>
<tr>
<td>Free T4 (ng/dl)</td>
<td>0.68 ± 0.261</td>
<td>0.95 ± 0.411*</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>22.56 ± 7.870</td>
<td>9.87 ± 7.210*</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.
*P<0.05 as compared to before treatment group

As evident from the table, there was a significant increase in FT3 and FT4 and a significant decrease in TSH in the pre-treatment and post-treatment phases. After 12 weeks of levothyroxine therapy, free T3 level increased significantly from 1.72 ± 0.683 pg/dl to 2.96 ± 0.916 pg/dl and free T4 level increased from 0.68 ± 0.261 ng/dl to 0.95 ± 0.411 ng/dl, both of which was statistically significant (P<0.05). Also, TSH level decreased significantly from 22.56 ± 7.870 μIU/ml to 9.87 ± 7.210 μIU/ml (P<0.05) after levothyroxine treatment in hypothyroid patients.

Effect of levothyroxine on serum hsCRP level
After 12 weeks of levothyroxine therapy, hsCRP level increased significantly from 3.26 ± 0.94 mg/l to 4.31 ± 1.78 mg/l which was statistically significant (P<0.05) as compared to before treatment (Table 2).
Table 2. Effect of Levothyroxine treatment on mean serum hsCRP levels in hypothyroid patients (n=40).

<table>
<thead>
<tr>
<th></th>
<th>Before treatment (n=40)</th>
<th>After treatment (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP (mg/L)</td>
<td>3.26 ± 0.94</td>
<td>4.31 ± 1.78*</td>
</tr>
</tbody>
</table>

*P<0.05

Grading of Cardiovascular Risk according to hsCRP value.

Figure 1 shows the cardiovascular risk grading based on the levels of hsCRP in the study population. Out of 40 patients, 17 patients had hsCRP values of more than 3 mg/l before levothyroxine treatment making them high risk for the development of CVD. After treatment with levothyroxine for 12 weeks, it increased by 12 cases making it a total of 29 cases under high risk group. Out of 40 cases, 23 subjects had hsCRP values in the moderate risk group and zero cases were in low risk group before levothyroxine treatment. 12 moderate risk group patient shifted towards high risk group after levothyroxine treatment thereby increasing the number of high risk patients in the study population.

Effect of levothyroxine on serum GSH level

After 12 weeks of levothyroxine therapy, GSH level increased from 0.97 ± 2.43 to 1.36 ± 1.98 μM which was statistically not significant.
DISCUSSION
Hypothyroidism is a common endocrine disorder affecting the thyroid gland. It is associated with decreased basal metabolic rate, lower heat production (hypothermia) and decreased oxygen expenditure.[7] Our enzymes produce ROS in increased quantities in response to inflammation leading to oxidative stress. This higher oxidative burden in hypothyroid individuals leads to endothelial dysfunction. Cardiovascular risk in patients with hypothyroidism is usually attributed to an atherogenic lipid profile. Chronic inflammation is hallmark of atherosclerosis. The acute-phase reactant protein hsCRP is a sensitive and nonspecific marker of inflammation, infection and tissue damage. Raised hsCRP levels indicate the severity and presence of inflammation. Thus, hsCRP is increasingly being recognized as a significant cardiovascular event marker, primary as well as recurrent.[13] Although hypothyroidism is closely related to both oxidative stress and atherosclerosis, the results of association between hypothyroidism treatment with levothyroxine replacement therapy and its effect on hsCRP levels and oxidative stress parameters have been conflicting among the different trials. Thus, this study was planned to evaluate the levothyroxine effect on hsCRP and glutathione levels in hypothyroid patients in the setup of a tertiary care teaching hospital. In this study, 40 newly diagnosed patients of hypothyroidism were enrolled. The mean age of patients was 40.76 ± 11.05 years. Out of 40 patients, 85% were female. This corresponds to the well-known fact that thyroid disorders are more common in females as compared to males.[21] As expected, the serum levels of FT3 and FT4 increased significantly and TSH decreased significantly towards normal range with levothyroxine therapy.[22] It was observed that at the end of 12 weeks of levothyroxine therapy, hsCRP level increased from 3.26 ± 0.94 mg/l to 4.31 ± 1.78 mg/l which was statistically significant. Also, the total number of patients with hsCRP values more than 3 mg/l (high risk group) increased to 29 after levothyroxine therapy as compared to 17 patients before levothyroxine treatment. The patients from moderate risk group shifted towards high risk group after levothyroxine treatment. So, it was observed that there was a significant increase in level of hsCRP and an apparent increase in cases in high risk group for cardiovascular risk after treatment with levothyroxine. Some studies have been conducted previously to evaluate the association between levothyroxine treatment and
serum hsCRP levels, but they have different and inconclusive results. The study findings were supported by a study conducted by Aksoy et al. who also reported an increase in hsCRP levels after levothyroxine treatment, but the increase was not statistically significant.\cite{7} Also, a recent study by Dey et al. associated subclinical hypothyroidism with increased hsCRP levels.\cite{23} In contrast to this, Marchiori et al. observed no change in hsCRP levels after treatment with levothyroxine for twelve months.\cite{24} However, Bilgir et al., after 3 months of levothyroxine administration reported a decrease in hsCRP levels but it did not reach a statistically significant level.\cite{25} In contrast to the study finding of increase number of patients in high cardiovascular risk group, Nagasaki T. et al. reported a decrease in CRP levels after one year of levothyroxine treatment and an improvement in arterial stiffness in their study cohort.\cite{17}

This study observed an increase in serum reduced glutathione levels, from $0.97 \pm 2.43 \mu M$ at baseline to $1.36 \pm 1.98 \mu M$ after 12 weeks of levothyroxine treatment but the increase was not statistically significant. This suggests that levothyroxine treatment may further increase oxidative stress in a hypothyroid patient. Similar to the study results, the study by Becer et al. reported that after levothyroxine treatment there was a significant increase in total antioxidant status (TAS) and malondialdehyde (MDA) levels suggesting increased oxidative stress post levothyroxine treatment.\cite{15} Similarly, Tomella et al. observed significant increase in glutathione peroxidase level and levothyroxine treatment further increased oxidative stress.\cite{16} In contrast to these findings, Kebapcilar et al. reported no change in the level of thiobarbituric acid reactive substances with levothyroxine therapy in subclinical hypothyroidism.\cite{26} Chakrabarti et al. reported a statistically significant decrease in the levels of MDA suggesting a reduction in oxidative stress in hypothyroid patients post levothyroxine treatment.\cite{27}

Thus, an explanation for increase in oxidative stress and hsCRP levels in our study, could be that the administration of levothyroxine tries to bring the basal metabolic rate (BMR) towards normal range, which is below normal in hypothyroid individuals. Hence, it can be hypothesized that as BMR goes towards normal, it may increase oxidative stress and hsCRP, due to normalization of various enzymatic functions. Following levothyroxine treatment, there is a further increase in inflammation which can exacerbate oxidative stress in hypothyroid patients and both the factors can exert an additive effect to increase the risk of atherosclerosis and CVD in hypothyroid patients. This study has several potential limitations. The most important ones are small sample size and as well as short duration. The relief in the symptoms may take 3-6 months after restoration of TSH levels to normal. As this study is of 12 weeks’ duration only, further studies to see full effect of levothyroxine replacement therapy over six months to one-year period is needed to come to a rightful conclusion. Also, only one oxidative stress parameter has been considered in this study, so future studies analysing multiple oxidative stress parameters must be planned to gain more insight into this area.

CONCLUSION

In this study, significant increase in serum level of reduced glutathione and hsCRP was found and thus, the treatment with levothyroxine therapy may not protect patients from cardiovascular morbidity and mortality due to atherosclerosis. To conclude, it can be said that the positive clinical benefits of levothyroxine replacement are slow to appear and insufficient to counter the effect of oxidative stress and inflammation, thereby the potential cardiovascular risks. It is essential to look for other treatment strategies to counter this in hypothyroid patients. Also, further long duration prospective studies should be conducted to evaluate the effect of long term levothyroxine treatment on various oxidative stress and inflammatory markers.

REFERENCE


