Research Article

# Thyroid hormones - its influence on the level and activity of nitric oxide and inflammatory marker among women with hypothyroidism

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## Abstract

**Background:** Hypothyroidism is associated with hypo metabolic state and endothelial dysfunction; it results in the activation of nitric oxide synthase enzyme, which releases nitric oxide. Nitric oxide synthase derived peroxynitrite has a central component of inflammation. Inflammatory and autoimmune conditions cause synthesis of hs-CRP by liver. This study was done to compare and evaluate the correlation between thyroid function, nitric oxide and inflammatory status in hypothyroid subjects. **Material and Methods**: 50 cases each of Euthyroidism and Hypothyroidism were included in this study. Blood samples were drawn for the estimation of nitric oxide (NO), hs-CRP and thyroid profile in all the study subjects. **Results:** No values were significantly decreased in hypothyroid patients (n=50) compared to controls. (n=50) (p<.000) hs-CRP values were significantly increased in hypothyroid patients (n=50) compared to euthyroid controls (n=50). (p<.000) hs-CRP had a significant negative correlation with $ft_3$ and $ft_4$ and positive correlation with TSH.NO had a significant positive correlation with $ft_3$and$ft_4$and negative correlation with TSH. **Conclusion:** These findings suggest that raised hs-CRP and reduced NO levels in hypothyroidism could cause subclinical inflammatory state which could be a risk factor for atherosclerosis and CVD.

**Keywords:** Thyroid hormones, nitric oxide, hypothyroidism.

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## INTRODUCTION

In India burden of thyroid disease are huge[1,2] Hypothyroidism is associated with cardiovascular risk factors[3]and endothelial dysfunction[4] In Rotterdam Study, an English cross-sectional analysis of 1149 women with subclinical hypothyroidism had a higher prevalence of cardio vascular disease.[5] Nitric oxide (NO) is produced as the result of transformation of L-arginine to L-citruline by a family of enzymes known as NO synthase (NOS) which has central component of inflammation, it causes the synthesis of hs-CRP by liver.[6] NO also plays a role in the regulation of thyroid vascularity and blood flow and also an important regulator of vascular tone.[7,8] In hypothyroidism endothelial vascularity and integrity is compromised due to low $t_3$and $t_4$ levels resulting in endothelial dysfunction. NO levels are low in endothelial dysfunction.[9] Reduced bioavailability of NO is involved in the initiation, progression and complications of atherosclerosis.[10] Studying the NO level in hypothyroidism will lead to an understanding of its role in hypothyroidism individuals. As inflammatory biomarker, hs-CRP has been widely used for predicting atherosclerosis and cardiovascular disease[11], hs-CRP has a strong associated with cardiovascular events have been shownin some studies.[12,13] One study showed that low free thyroxine is associated with elevated hs-CRP[14], anotherstudy showed no difference in hs-CRP levels between patients with SCH and euthyroid individuals.[15]

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Recently hs-CRP has been shown it may affect NO pathway.[16] Determination of its level will help in understanding the amount of systemic inflammation in hypothyroid patients. Given the importance of NO and hs-CRP in the inflammatory and atherosclerotic events, the aim of the present study was to analyse whether hypothyroidism causes change in NO and hs-CRP levels.

## MATERIAL AND METHODS

The present study was conducted in the Department of Biochemistry, Manakula Vinayagar Medical College and Hospital, in collaboration with Department of medicine from May 2013 to March 2014. Females attending the medicine OPD and female patients in the medicine ward were enrolled for this study.50 patients with hypothyroidism were taken as cases. The diagnosis of hypothyroidism was made by the presence of TSH levels > 4.5 µIU/ml (reference value British thyroid foundation). 50 age and sex matched healthy volunteers with normal thyroid hormone profile were taken as controls. Patients with history of vascular disease, cardiac disease, diabetes mellitus and other inflammatory conditions which interfere with hs-CRP and NO values were excluded from the study. After getting approval from Institute Human Ethical Committee. After explaining the nature of the study, informed consent was obtained from the study subjects. About 5 ml of blood sample was collected from all the study subjects in a clot activator tube. Serum was separated by centrifugation and analyzed for required parameters.

### Estimation of study Parameters

1. Serum $fT_3$ is measured by competitive analogue immunoassay method.
2. Serum $fT_4$ is measured by solid phase enzyme labeled chemiluminescent competitive immunoassay method.
3. Serum TSH is measured by solid phase two site chemiluminescent immunometric assay method. Standard kits obtained from Siemens Healthcare Diagnostics Ltd were used on an Immulite fully automated analyser from Siemens for the estimation of thyroid parameters.
4. Serum NO is measured by Griess Diazotization reaction method using spectrophotometer.Kit manufacturer-Molecular Probes, U.S.A.
5. Serum hs-CRP is measured by Turbidimetric immunoassay using turbidometry analyser. Kit manufacturer-Tulip diagnostic Goa.

### Statistical Analysis

Results were shown as mean± S.D. Comparison of parameters between the cases and controls was done using student's test. A p-value of less than 0.05 was considered statistically significant. Correlation analysis was done using Pearson's Correlation. All calculations were performed using the SPSS software version 16.

## RESULTS

In this study 50 hypothyroid cases and 50 euthyroid controls were included, after full filling the exclusion criteria. Euthyroid and hypothyroid subject's thyroid profile, NO and hs-CRP levels and their statistical differences are shown in table 1.

**Table 1:** Thyroid profile between euthyroid controls and hypothyroid cases

| Parameters | Controls(n=50) | Cases(n=50) | p value |
|---|---|---|---|
| $fT_3$(pg/ml) | 2.91±.50 | 1.36±.34 | .000 |
| $fT_4$(ng/dl) | 1.13±.27 | .51±.17 | .000 |
| TSH(µIU/ml) | 1.55±1.1 | 35.2±26.5 | .000 |

The values are expressed as Mean ± SD. n is the number of patients.

p value of <0.05 is considered statistically significant

**Table 2:** Comparison of NO and hs-CRP levels between euthyroid controls and hypothyroid cases

| Parameters | Controls(n=50) | Cases(n=50) | p value |
|---|---|---|---|
| hs-CRP(mg/L) | 3.2±.34 | 7.5±.79 | .000 |
| NO(µmol/L) | 29.3±.1.5 | 21.5±.94 | .000 |

The values are expressed as Mean ± SD. n is the number of patients.

p value of <0.05 is considered statistically significant

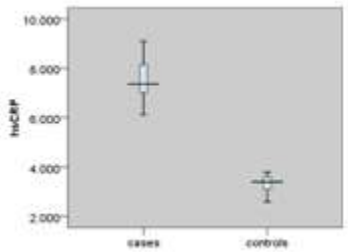



**Figure 1:** Boxplot: comparison of hs-CRP levels between cases and controls

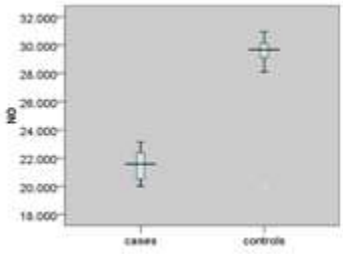


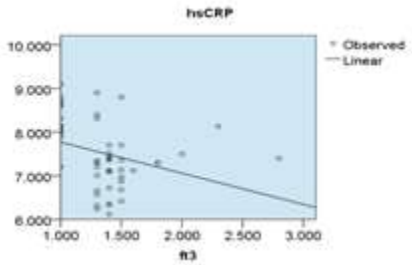

**Figure 2:** Box plot: comparison of NO levels between cases and controls

From table 1, it is evident that there is a significant difference between the means of TSH in the hypothyroid cases and euthyroid controls (35.2±26.5µIU/ml)versus (1.55±1.1µIU/ml)(p<. 000),from table 2,it is evident that there is significant difference between the means of hs-CRP in the hypothyroid cases and euthyroid controls (7.5±.79mg/L)versus (3.2±.34mg/L) (p<. 000), significant difference between the means of NO in the hypothyroid cases and euthyroid controls (21.5±.94µmol/L)versus (29.3±.1.5µmol/L) (p<. 000),significant difference between the means of $fT_3$ in the hypothyroid cases and euthyroid controls (1.36±.34pg/ml)versus (2.91±.50pg/ml) (p<. 000),significant difference between the means of $fT_4$ in the hypothyroid cases and euthyroid controls (.51±.17ng/dl)versus (1.13±.27ng/dl) (p<. 000) Figure-1 shows the hs-CRP values in cases: median is 7.36mg/L, minimum range is 6.12mg/L, maximum range is 9.10mg/L.hs-CRP values in controls: median is 3.4mg/L, minimum range is 2.60mg/L,maximum range is 3.80mg/L Figure-2 shows the NO values in cases median is 21.57 µmol/L, minimum range is 20.10µmol/L, maximum range is 23.15µmol/L.NO values in controls: median is 29.67µmol/L, minimum range is 19.94µmol/L, maximum range is 30.94µmol/L.

**Table 3:** Correlation between thyroid hormones, hs-CRP and NO among cases

| Parameters (n=50) | hs-CRP | | NO | |
|---|---|---|---|---|
| | r value | p value | r value | p value |
| $ft_3$ | -.306** | .031 | .386* | .006 |
| $ft_4$ | -.402 | .004 | .399** | .004 |
| TSH | .752 | .000 | .-686** | .000 |

**Correlation is significant at the. 01 level.*Correlation is significant at the. 05 level.



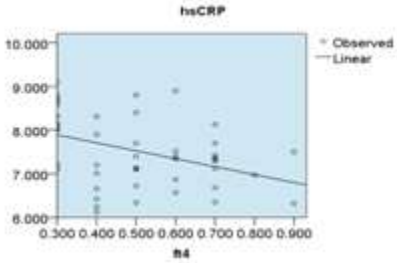
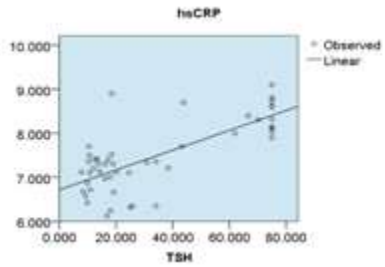




**Figure 3:** Shows a significant negative correlation between fT3 (pg/ml) and hs-CRP (mg/L) of the cases (r = -.306, p = .031)

**Figure 4:** Shows a significant negative correlation between fT4 (ng/dl) and hs-CRP (mg/L) of the cases (r = -.402, p = .004)



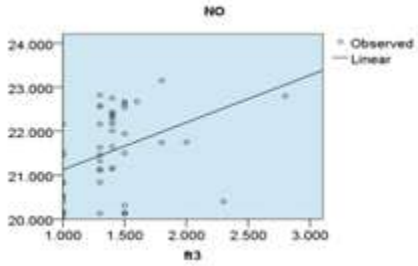
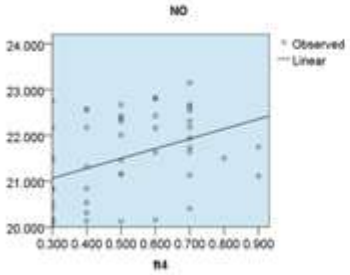




**Figure 5:** Shows a significant positive correlation between TSH (µIU/ml) and hs-CRP (mg/L) of the cases (r =. 752, p =. 000)

**Figure 6:** shows a significant positive correlation between fT3 (pg/ml) and NO (µmol/L) of the cases (r =. 386, p =. 006)



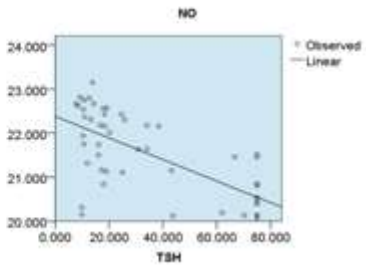




**Figure 7:** Shows a significant positive correlation between fT4 (ng/dl) and NO (µmol/L) of the cases (r =. 399, p =. 004)

**Figure 8:** Shows a significant negative correlation between TSH (µIU/ml) and NO (µmol/L) of the cases (r =.-686, p =. 000)

## DISCUSSION

In India 42 million people suffer from thyroid diseases.[17] Hypothyroidism is associated with risk factors like atherosclerotic cardiovascular disease, low grade inflammation and endothelial dysfunction.[3, 4,18,19] NO is produced by the NOS enzyme in response to the vascular insult, NO levels are reduced in hypothyroidism.[4] The effects of thyroid status on NOS gene expression in the rats shows it is reduced in hypothyroidism.[20] Every time NO and superoxide collide, they form peroxynitritenon-enzymatically and very rapidly. Under proinflammatory conditions, simultaneous production of superoxide and NO can be strongly activated to increase production 1000-fold, which will increase the formation of peroxynitrite by a1000, 000-fold.[21] Peroxynitrite contributes to the endothelial dysfunction.[22] NO is converted to nitrite under aerobic conditions. Nitrite is proven as an endogenous signal molecule and regulator of gene expression and also as a diagnostic marker of cardiovascular disease.[4] In our study, we observed decreased values of NO in cases when compared with controls. Previous studies have shown similar results.[23,24] When NO is correlated with thyroid hormones there was a positive correlation with $ft_3$and$ft_4$, negative correlation with TSH. It shows when then the TSH level rises NO level decreases. The reason for the decreased NO levels in our study could be due to hypothyroidism which causes vascular changes, endothelial dysfunction and reduced metabolic turn over, the expression of NOS synthase enzyme is decreased which effects L-arginine-NO system assuch leading to the decreased production of NO. Studies have shown 70%-90% of nitrites in plasma come from endothelial nitric oxide synthase (eNOS) activity.[25] NOS-derived peroxynitrite induces inflammatory conditions to release interleukin-6 and other cytokines that trigger the synthesis of hs-CRP by liver. Elevated hs-CRP can deprive the blood supply in vascular endothelium and cause ischemia in a complement-dependent fashion this has been demonstrated in animal models.[26] In addition, hs-CRP may aggravate the atherothrombotic process with activation of complement and monocytes/ macrophages.[27] In our study we observed an increase level of hs-CRP in hypothyroid cases compare to controls. Previous studies have shown similar results. In one study subjects with subclinical hypothyroidism had significantly higher levels of serum hs-CRP, when compared to same parameters of controls. Further, a significant positive correlation was observed between TSH and hs-CRP.[28] In our study when thyroid hormones were correlated with hs-CRP there was a positive correlation between TSH and hs-CRP.$ft_3$and$ft_4$ had a negative correlation. These results show that when severity of hypothyroidism increases level of hs-CRP also rises which an inflammatory marker is. The reason for the raised hs-CRP levels in our study could be due to hs-CRP released from liver as a response to inflammation. Hypothyroidism could promote chronic subclinical inflammation which rises Interleukin-6 levels leading to raised hs-CRP levels. Raised hs-CRP could promote atherosclerosis directly.

## CONCLUSION

Our findings suggests that abnormal thyroid hormone levels regulate the activity and level of NO and hs-CRP which could lead to complication like atherosclerosis and Cardio vascular diseases.

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