



LIPID PROFILE AND HIGH SENSITIVITY C- REACTIVE PROTEIN (HS-CRP) IN WOMEN WITH SUBCLINICAL HYPOTHYROIDISM: BEFORE AND AFTER THERAPY

Medical Biochemistry

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ABSTRACT

Background: Subclinical hypothyroidism (SCH) is defined as an asymptomatic condition with raised levels of thyroid-stimulating hormone (TSH) and normal levels of free thyroid hormones (FT3 and FT4). The effect of subclinical hypothyroidism and its therapy on risk factors for cardiovascular disease remains controversial. **Objective:** To study serum lipid profile and high-sensitivity C-reactive protein (HS-CRP) levels in women with subclinical hypothyroidism, before and after therapy. **Methods:** For this study, 50 newly diagnosed and untreated subclinical hypothyroid (SCH) female patients between the age group 18-45 years and age matched 50 healthy euthyroid female volunteers as controls were selected and enrolled in the study. Serum lipid profile and high-sensitivity C-reactive protein (HS-CRP) were evaluated in controls and in cases, before and after therapy (irrespective of therapy). **Results:** Total cholesterol, LDL- cholesterol, triglyceride (TG) and HS-CRP levels were significantly increased whereas HDL- C was found to be decreased in the SCH group as compared to the control group. TSH was significantly positively correlated with total cholesterol, LDL-C, TG, and negatively correlated with HDL-C in SCH patients. Total cholesterol, LDL-C, TG and HS-CRP levels were significantly decreased, and HDL-C level was found to be increased in SCH patients after therapy as compared to before therapy. **Conclusion:** Dyslipidemia and a state of low-grade inflammation as indicated by elevated levels of HS-CRP starts in the early stages of hypothyroidism predicting an increased risk of CVD. Early screening and proper treatment may help to reduce the risk of cardiovascular disease and improve the quality of life in these patients.

KEYWORDS

Subclinical Hypothyroidism, Lipid Profile, High Sensitivity C-Reactive Protein.

INTRODUCTION

Hypothyroidism is the most common thyroid disorder in India, affecting one in ten adults. The prevalence of hypothyroidism in India is 11%, compared with only 2% in the UK and 4-6% in the USA. The prevalence of hypothyroidism increases with age and is nearly ten times more in women than in men. Iodine deficiency has been considered the most common cause of hypothyroidism. Chronic autoimmune thyroiditis (Hashimoto's disease) is the leading cause of hypothyroidism in iodine-sufficient areas [1-3]. Hypothyroidism is a graded phenomenon extending from mild or sub-clinical to severe overt hypothyroidism. Subclinical hypothyroidism (SCH) is defined as an asymptomatic condition with raised levels of thyroid-stimulating hormone (TSH) and normal levels of free thyroid hormones (FT3 and FT4) [4]. The prevalence of subclinical hypothyroidism has been reported between 8.02% -19.3% of the population in various studies across India [5,6]. Thyroid hormones play an essential role in regulating many metabolic processes, especially the metabolism of glucose and lipids. Hypothyroidism is a well-known cause of obesity and dyslipidemia. It has been reported that even slightly elevated serum TSH levels are significantly associated with an increase in the occurrence of obesity [7,8]. Although asymptomatic, various studies reported dyslipidemia in patients with subclinical hypothyroidism (SCH) as increased total as well as LDL cholesterol and triglyceride (TG) levels that result in an increased risk of cardiovascular disease in these patients and showed that SCH is an independent risk factor for myocardial infarction (MI) and atherosclerosis in elderly women [9,10]. Whereas, other few studies did not find any significant difference in the levels of these lipid parameters between SCH patients and controls [11-13]. Some studies have reported beneficial effects of thyroxine therapy on lipid parameters in subclinical hypothyroid patients [14-16]. However, others did not find a significant change in lipid parameters in subclinical hypothyroid patients after therapy compared to before therapy [11-12].

Recent data suggest that inflammation plays a central role in the pathogenesis of atherosclerotic cardiovascular disease. Numerous inflammatory markers have emerged as an independent risk factor for cardiovascular disease. High-sensitivity C-reactive protein (HS-CRP), a marker of systemic inflammation is now considered a strong

predictor of future cardiovascular events [11]. Elevated levels of inflammatory markers such as HS-CRP are found to be directly related to the risk of myocardial infarction [17]. Nowadays HS-CRP has been widely used as an inflammatory biomarker to assess the cardiovascular risk in healthy subjects as well as in people with various disorders. Previous studies have shown the state of low-grade inflammation, as indicated by elevated levels of HS-CRP in subclinical and overt hypothyroidism [12,14], whereas another study observed no significant difference as compared to controls [13]. Few studies showed a significant reduction of HS-CRP levels [12,14], whereas other studies did not find a significant difference in HS-CRP levels after thyroxine therapy in patients with subclinical and overt hypothyroidism [18,19]. Limited and conflicting data is available about the status of dyslipidemia and low-grade inflammation on the restoration of euthyroidism, after therapy in patients with subclinical hypothyroidism. It remains controversial whether L-thyroxine therapy reduces the risk of cardiovascular disease in patients with hypothyroidism. Hence, we planned to study serum lipid profile and high-sensitivity C-reactive protein (HS-CRP) levels in women with subclinical hypothyroidism, before and after therapy.

MATERIAL AND METHODS

The present cross-sectional study, as a part of Ph.D. work, was conducted in the Department of Biochemistry, Govt. Medical College and Hospital, Chh. Sambhajinagar (Aurangabad) during the period from April 2012 to May 2014. For this study, newly diagnosed and untreated 50 subclinical hypothyroid women from the outpatient Department of Medicine were selected and enrolled in the study as cases. All the anthropometric, clinical, and biochemical parameters were evaluated and compared in these patients before therapy and 4-6 weeks after therapy (irrespective of therapy), on the restoration of euthyroidism. Age-matched 50 healthy euthyroid female volunteers were selected and included in the study as controls. The study protocol was approved by the institutional ethics committee of Govt. Medical College and Hospital, Chh. Sambhajinagar (Aurangabad). Informed consent was obtained from all cases and controls before participating in the study. The selection of cases and controls was done based on inclusion and exclusion criteria.

Inclusion Criteria

All the cases and controls belong to the age group 18 to 45 years.

Exclusion Criteria

Women suffering from diabetes, polycystic ovarian disease, liver or renal disorders, other systemic illnesses, intake of oral contraceptive pills, statins, and other medication that alter thyroid function and lipid levels were excluded from the study. Pregnancy and menopause also accounted for exclusion from the study.

Subclinical Hypothyroidism: is defined as patients with increased TSH levels ($>5.45\text{mIU/ml}$) and free T3 and T4 levels within their respective reference ranges.

Euthyroidism: Patients with TSH, FT3, and FT4 levels within their respective reference range were considered euthyroid.

Anthropometric and Clinical Measurements

- Waist circumference (cm): was measured at the midpoint of the distance between the lowest rib and the iliac crest using a standard measuring tape.
- Blood Pressure (mmHg): was measured by using a standard sphygmomanometer on the left upper arm with the patient in a sitting position.

Biochemical Investigations

In the case of all study participants, under all aseptic conditions, 12-hour fasting venous blood was collected in plain blood collection tubes and serum separated by centrifugation was used for the estimation of biochemical parameters. Estimation of serum Thyroid profile (TSH, FT3, and FT4) and High sensitivity-C reactive protein (HS-CRP) was done by Chemiluminescence Immunoassay (CLIA) using Acculite CLIA microwells assay kits from Monobind INC., Lake Forest, CA 92630, USA. Serum total cholesterol (CHOD-POD), triglyceride (GPO), and HDL-C (PVS/PEGME) were estimated on XL-640 fully automated clinical chemistry analyzer, Transasia Pvt. Ltd. by using commercially available kits from Erba diagnostics. LDL-C was calculated using Friedward's formula ($\text{LDL-C} = \text{Total-C} - (\text{HDL} + \text{TG}/5)$) [20].

Statistical Analysis

Data was expressed as mean $\pm$ SD. A comparison of parameters between cases and controls was done using an unpaired 't-test'. Comparison of mean values of parameters before therapy and after therapy was done using the paired 't-test'. Pearson's correlation coefficient (r) was used to assess the relationship between different variables. Statistical analysis was performed using free Open-Epi software, Microsoft Word, and Excel worksheets. The value $P > 0.05$ was considered non-significant and $P < 0.05$ was considered as significant.

RESULTS

Table-1 shows the comparison of parameters between control group and subclinical hypothyroid (SCH) group. FT3 and FT4 levels were significantly decreased ($P < 0.05$) whereas TSH was significantly increased ($p < 0.0001$) in the subclinical hypothyroid group as compared to the control group. Similarly, total cholesterol, LDL-cholesterol, triglyceride and HS-CRP levels were significantly increased in the SCH group as compared to the control group. HDL-cholesterol was found to be decreased in the SCH group as compared to the control group but it was not statistically significant.

Table-2 shows the correlation between TSH and biochemical parameters in subclinical hypothyroidism. TSH was significantly positively correlated with diastolic blood pressure ($P < 0.05$), waist circumference ($P < 0.01$), total cholesterol ($P < 0.05$), LDL-C ($P < 0.01$), TG ($P < 0.05$) and HS-CRP ($P < 0.05$). On the other hand, TSH was found to be significantly negatively correlated with HDL-C ($P < 0.01$).

Table-3 shows the comparison of various clinical and biochemical parameters in SCH patients before and after therapy. FT3 and FT4 levels were significantly increased ($P < 0.05$; $P < 0.001$) and TSH was significantly decreased in SCH ($P < 0.001$) after therapy as compared to those before therapy. Waist circumference and systolic blood pressure were not significantly changed but diastolic blood pressure was significantly decreased in SCH patients after therapy as compared to before therapy ($P < 0.001$). Total cholesterol ($P < 0.0001$), LDL-C ($P < 0.0001$), TG ($P < 0.001$) and HS-CRP ($P < 0.01$) levels were significantly decreased in SCH patients after therapy as compared to

before therapy. On the other hand, HDL level was found to be increased in SCH patients after therapy as compared to before therapy but it was not statistically significant.

Table- 1: Baseline Characteristics of Study Groups

Parameters	Control (n=50)	Subclinical hypothyroid (n=50)	P
Age (years)	36.66 $\pm$ 6.78	37.04 $\pm$ 5.51	0.7591 NS
FT3 (pg/ml)	2.82 $\pm$ 0.30	2.66 $\pm$ 0.39	$P < 0.05$
FT4 (ng/dl)	1.34 $\pm$ 0.15	1.28 $\pm$ 0.13	$P < 0.05$
TSH ($\mu\text{IU/ml}$)	2.17 $\pm$ 0.71	12.45 $\pm$ 3.24	$P < 0.0001$
Waist circumference (cm)	80.4 $\pm$ 5.11	81.87 $\pm$ 4.71	$P = 0.1379$ NS
Systolic blood pressure (mmHg)	120.8 $\pm$ 5.88	121.44 $\pm$ 5.36	$P = 0.5708$ NS
Diastolic blood pressure (mmHg)	80.04 $\pm$ 6.12	82.72 $\pm$ 4.97	$P < 0.05$
Total cholesterol (mg/dl)	175.6 $\pm$ 19.13	191.18 $\pm$ 20.95	$P < 0.01$
HDL-C (mg/dl)	47.35 $\pm$ 7.6	46.75 $\pm$ 6.6	$P = 0.6743$ NS
LDL-C (mg/dl)	106.4 $\pm$ 21.76	116.28 $\pm$ 22.97	$P < 0.05$
Triglyceride (mg/dl)	112.7 $\pm$ 21.39	140.67 $\pm$ 18.43	$P < 0.0001$
HS-CRP ($\mu\text{g/ml}$)	1.97 $\pm$ 0.55	2.46 $\pm$ 0.74	$P < 0.001$

values were expressed as mean $\pm$ SD, NS: non-significant, $P < 0.05$: considered significant

Table-2: Correlation Between TSH and Various Clinical and Biochemical Parameters in Subclinical Hypothyroidism

Parameter	TSH-correlation coefficient(r)	
	r-value	p-value
Waist circumference	0.3848	$P < 0.01$
Systolic blood pressure	0.0719	NS
Diastolic blood pressure	0.3126	$P < 0.05$
Total cholesterol	0.3579	$P < 0.05$
HDL-C	-0.486	$P < 0.01$
LDL-C	0.421	$P < 0.01$
TG	0.2827	$P < 0.05$
HS-CRP	0.2824	$P < 0.05$

NS: non-significant ($P > 0.05$) $p < 0.05$: considered significant

Table- 3: Clinical and Biochemical Parameters in Subclinical Hypothyroidism: Before and After Therapy

Parameter	Subclinical hypothyroidism		p
	Before therapy (n=50)	After therapy (n=50)	
FT3 (pg/ml)	2.66 $\pm$ 0.39	2.78 $\pm$ 0.38	$P < 0.05$
FT4 (ng/dl)	1.28 $\pm$ 0.13	1.33 $\pm$ 0.14	$P < 0.001$
TSH ($\mu\text{IU/ml}$)	12.45 $\pm$ 3.24	2.93 $\pm$ 0.46	$P < 0.0001$
Waist circumference (cm)	81.87 $\pm$ 4.71	81.59 $\pm$ 4.56	NS
Systolic blood pressure (mmHg)	121.44 $\pm$ 5.36	121.20 $\pm$ 3.61	NS
Diastolic blood pressure (mmHg)	82.72 $\pm$ 4.97	80.84 $\pm$ 3.25	$P < 0.001$
Total cholesterol (mg/dl)	191.18 $\pm$ 20.95	182.30 $\pm$ 20.78	$P < 0.0001$
HDL-C (mg/dl)	46.75 $\pm$ 6.60	48.64 $\pm$ 7.46	NS
LDL-C (mg/dl)	116.28 $\pm$ 22.97	106.60 $\pm$ 22.95	$P < 0.0001$
Triglyceride (mg/dl)	140.67 $\pm$ 18.43	135.47 $\pm$ 14.19	$P < 0.001$
HS-CRP ($\mu\text{g/ml}$)	2.46 $\pm$ 0.74	2.34 $\pm$ 0.65	$P < 0.01$

values were expressed as mean $\pm$ SD, NS: non-significant, $P < 0.05$: considered significant

DISCUSSION

Hypothyroidism is the most common endocrine disorder in the general population, especially in women. Thyroid hormones influence nearly all major metabolic pathways and lipid metabolism is more influenced by thyroid hormones. In our study (Table-1), we found that the mean values of total cholesterol ($p < 0.01$), LDL-C ($p < 0.05$), and Triglyceride (TG) ($p < 0.0001$) were significantly elevated in the subclinical hypothyroid group compared to the control group. These findings are in agreement with the previous studies [16]. In contrast to

these, few studies did not find any significant difference in the levels of these lipid parameters between SCH patients and controls [11-13]. Thyroid hormones, especially T3 induce LDL- receptor gene expression in the liver, enhancing LDL clearance. Therefore, in hypothyroidism, increased levels of total cholesterol and LDL-C are observed due to the reduced activity of LDL receptors. The elevation of triglycerides may be due to the decreased lipoprotein lipase (LPL) activity observed in hypothyroidism that results in poor clearance of TG-rich lipoproteins as well as endogenous and exogenous triglycerides from circulation. Thyroid hormones affect cholesteryl ester transfer protein and hepatic lipase activity, which are found to be decreased in hypothyroidism, which results in a reduced transport of cholesteryl esters from HDL 2 to VLDL and intermediate density lipoprotein (IDL) as well as reduced transport of HDL -2 to HDL- 3 with consequent changes in total HDL and HDL subfractions levels [16]. In our study, HDL cholesterol levels were found to be decreased in the subclinical hypothyroid patients but not statistically significant when compared with the control group. These findings are in agreement with some studies [12-16], however, few studies reported significantly increased levels of HDL-C in subclinical hypothyroid patients [16]. Hypothyroidism has been recognized as a cause of secondary hypertension. In the present study (Table-1), we observed that systolic blood pressure (SBP) was not changed significantly ($p>0.05$), whereas diastolic blood pressure (DBP) was found to be significantly increased ($p<0.05$) in the subclinical hypothyroid group compared to the control group. These findings are in agreement with previous study, in which they found a strong association between elevated diastolic blood pressure and hypothyroidism [21]. Similarly, the Rotterdam study reported SCH to be a strong independent risk factor for myocardial infarction in elderly women [9]. We also observed that (Table-2), waist circumference, total cholesterol, LDL-C, Triglyceride (TG), as well as diastolic blood pressure were significantly positively correlated with TSH, whereas HDL-C was found to be negatively correlated with TSH in SCH patients. In the present study, (Table-3), we observed that total cholesterol ($p<0.0001$), LDL-C ($p<0.0001$), and Triglyceride (TG) ($p<0.001$), levels were significantly decreased in subclinical hypothyroid groups after therapy compared to those before therapy. On the other hand, HDL-C level was found to be increased in subclinical hypothyroid patients after therapy as compared to before therapy but it was not statistically significant. Our findings are in agreement with the previous studies that reported beneficial effects of thyroxine therapy on lipid parameters in subclinical and hypothyroid patients [14-16]. However, in contrast to our findings, other studies did not find a significant change in lipid parameters in subclinical hypothyroid patients after therapy compared to before therapy [11-12]. In the present study (Table-3), we found that systolic blood pressure was not changed significantly ($p>0.05$) but, diastolic blood pressure was significantly decreased in SCH patients after therapy as compared to that before therapy ($P<0.001$). In agreement with our findings, a study conducted recently in Serbia reported a significant reduction in both systolic and diastolic blood pressure in SCH patients after therapy compared to before therapy [22]. The relationship between hypothyroidism and atherosclerosis is well known according to epidemiological studies and biochemical observations. There has been evidence that suggests an important role of inflammation in all phases of the atherosclerotic process. It has been stated that HS-CRP is a marker of systemic inflammation and a strong predictor of future cardiovascular events [12]. In the present study (Table-1), we found significantly elevated ($p<0.001$) levels of HS-CRP in subclinical hypothyroid patients as compared to the control group. We also observed that (Table-2), HS-CRP levels were significantly positively correlated with TSH in subclinical hypothyroid patients. These findings are in agreement with previous studies which reported significantly elevated levels of HS-CRP in SCH patients [12,14]. It has been shown that HS-CRP is not only produced by the liver but also from atherosclerotic plaques activated by vascular cells, suggesting its role in systemic and tissue inflammation [23]. In contrast to our results, few studies [11,24], reported that no significant differences were observed in terms of HS-CRP levels in SCH and control groups. In our study (Table-3), we observed that HS-CRP levels were significantly reduced in subclinical hypothyroid patients after therapy as compared to before therapy ($P<0.01$). Similar findings were also reported by the previous studies in which they observed that levothyroxine replacement therapy significantly reduced HS-CRP levels in SCH patients [12, 14]. In contrast to our findings, other few studies reported that levothyroxine therapy did not affect HS-CRP levels in SCH patients [18, 19,24]. The findings in our study suggest that dyslipidemia as well as the state of low-grade inflammation as

seen by elevated levels of HS-CRP, both starts in the early stages of hypothyroidism. This might indicate a possible linkage between hypothyroidism and risk factors for cardiovascular disease. Similarly, our study demonstrated decreased levels of total cholesterol, LDL-C, triglyceride (TG) and HS-CRP after therapy compared to before therapy, which could contribute to reduced risk of cardiovascular disease (CVD) in these patients.

CONCLUSION

Finally, we conclude that dyslipidemia as well as the state of low-grade inflammation as seen by elevated levels of HS-CRP should be considered in newly diagnosed women with subclinical hypothyroidism. Early screening may help in proper treatment and management which may reduce the risk of cardiovascular disease and improve the quality of life in these patients. Further long-term follow-up studies are required to confirm whether these short-term benefits will translate into a significant reduction in the risk of CVD.

REFERENCES

- Chubb SA, Davis WA, Davis TM. Interactions among thyroid function, insulin sensitivity and serum lipid concentrations: The Fremantle diabetes study. *J Clin Endocrinol Metab*. 2005; 90:5317-20.
- Sanjeet Bagechi. Hypothyroidism in India: more to be done. *The Lancet Diabetes-Endocrinology*. 2014; 2:778.
- Jaime P.Almandoz, Hossein Gharib. Hypothyroidism: Etiology, Diagnosis and Management. *Med Clin N Am*. 2012; 96:203-221.
- Mansourian AR. The state of serum Lipid profiles in sub-clinical Hypothyroidism: A review of the literature. *Pakistan Journal of Biological Sciences* 2010; 13 (11): 556 – 562.
- Marwaha RK, Tondon N, Ganie MA, Kanwar R, Sastry A, Garg MK, et al. status of thyroid function in Indian adults: two decades after universal salt iodization. *J Assoc Physicians India*. 2012; 60:32-36.
- Karla S, Kumar A, Jarhyan P, Unnikrishnan AG. Indices of thyroid epidemiology. *Indian J Endocrinol metabol*. 2015; 19:844-47.
- Surender Kumar. Hyperthyroidism: Overview & management. *Gastroenterology*. 2004; Vol. 8(2): 84- 88.
- Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. *J Clin Endocrinol Metab*. 2003; 88:2438-2444.
- Hah AE, Pols HAP, Visser TJ, Drexhage HA, Hofman A, Witterman JCM. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: The Rotterdam study. *Ann Intern Med*. 2000; 132:270-278.
- Rafael Luboshitzky, Ariel Aviv, Paula Herer and Lena Lavie. Risk Factors for Cardiovascular Disease in Women with Subclinical Hypothyroidism. *Thyroid* 2002; 12(5):421-425.
- Baris Akinci, Abdurrahman Comlekci, Serkan Yener, Tevfik Demir, Firat Bayraktar, Faize Yuksel, Sena Yesil. The alteration of serum soluble CD40 ligand levels in overt and subclinical hypothyroidism. *HORMONES* 2007; 6(4):327-333.
- Nehad A. El-Nashar, Nahla F. Khattab, Laila M. Anis. Effect of Thyroxine Replacement Therapy on the Levels of Serum soluble Cd40 Ligand and Other Biochemical cardiovascular Risk Markers in Patients With hypothyroidism. *Med.J. Cairo Univ*. 2010; 78(1):517-526.
- Rafael Luboshitzky, Paula Herer. Cardiovascular risk factors in middle-aged women with hypothyroidism. *Neuroendocrinology Letters* 2004; 25(4):262-266.
- Orhan Kursat Poyrazoglu, Yusuf Ozkan, Mehmet Ozden, Ramis Colak, Goksel Ozalp, and Emir Dönder. L-Thyroxine Treatment of Patients with Subclinical Hypothyroidism Reduce Inflammation. *The Open Endocrinology Journal*, 2009; 3:34-37.
- Amit Saxena, Pragati Kapoor, Shikha Saxena and A K Kapoor. Effect of levothyroxine therapy on dyslipidemia in hypothyroid patients. *Internet Journal of Medical Update*. 2013; 8(2):39-49.
- Sarika Singh, Purmina Dey Sarkar. Serum lipids, tHcy, hs-CRP, MDA and PON-1 levels in SCH and overt hypothyroidism: effect of treatment. *Acta Biomed* 2014; Vol. 85(2): 127-134.
- Miller M, Zhan M, Havas S. High attributable risk of elevated C-reactive protein level to conventional coronary heart disease risk factors: the Third National Health and Nutrition Examination Survey. *Arch Intern Med*. 2005; 165: 2063-2068.
- Ronit Koren Peleg, Shai Efrati, Carlos Benbassat, Marina Fygenzo, Ahuva Golik. The effect of levothyroxine on arterial stiffness and lipid profile in patients with subclinical hypothyroidism. *Thyroid* 2008; 18(8):825-30.
- A Perez, JM Cubero, N Sucunza, E Ortega, R Arcelus, J Rodriguez-Espinosa, et al. Emerging cardiovascular risk factors in subclinical hypothyroidism: Lack of change after restoration of euthyroidism. *Metabolism*, 2004; 53(11):1512-1515.
- W.T. Friedwald, R.I. Levy, and D.S. Fredrickson. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry*. 1972; 18 (6): 499-502.
- Saito I, Saruta T. Hypertension in thyroid disorders. *Endocrinol Metab Clin North Am*. 1994; 23:379-386.
- Milena S Pandre, Andelka Ristic, Vanja Kostovski, Marko Stankovic, Vladimir Antic, Jelena Milin-Lazovic, Jasmina Ciric. The effect of Early Substitution of Subclinical Hypothyroidism on Biochemical Blood Parameters and the quality of Life. *J Med. Biochem*. 2017; 36(2):127-136.
- Montecucco F, Mach F. New evidence for C-reactive protein deposits in the arterial intima as a cardiovascular risk factor. *Clin Interv. Aging*. 2008; 3(2):341-349.
- Duygu Yazgan Aksoy, Nese Cinar, Ayla Harmanci, Jale Karakaya, Bulent Okan Yildiz, Aydan Usman, et al. Serum resistin and high- sensitive CRP levels in patients with subclinical hypothyroidism before and after L-thyroxine therapy. *Med Sci. Monit*. 2013; 19: 210-215.