Original Research Paper Medical Science

Comparison of Haematological Parameters in Untreated & Treated Primary Hypothyroid Patients in Shivamogga District

*** Dr.Vinit Anand** Associate Professor, Department of Pathology, Subbaiah Institute of Medical Sciences, Shivamogga * Corresponding author

Dr.Sridevi .V Associate Professor, Department of Biochemistry, Subbaiah Institute of Medical Sciences, Shivamogga

ABSTRACT

***Backgroun**d Thyroid hormones have a crucial role in metabolism and proliferation of blood cells. Thyroid dysfunction induces different effects on blood cells such as anaemia, erythrocytosis, leukopenia and thrombocytopenia and in rare cases pancytopenia. It also alters RBC indices.*

***Aims and objectives:** To estimate blood cell counts and blood cell indices in newly diagnosed Primary hypothyroid patients and to evaluate the efficacy of the L-thyroxine treatment on the same in Shivamogga population.*

***Materials and methods:** We measured Red blood cell count (RBC), White blood cells / Total leukocyte count (TLC), Differential leukocyte count (DLC), platelet (PLT) count, blood cell indices like haemoglobin [Hb] concentration, mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC], hematocrit (HCT) / Packed cell Volume (PCV) and Red cell width distribution (RDW) in 100 newly diagnosed Primary hypothyroid patients & compared it with the euthyroid controls. The same patients were revaluated after treatment with L-thyroxine at the end of three months. Statistical analysis was done using independent T test. Pearson's correlation coefficient test was done to establish the relationships between the parameters.*

***Results:** RBC count was significantly decreased in newly diagnosed Primary hypothyroid patients when compared to euthyroid subjects. There was no significant difference in TLC, DLC and Platelet count. Blood cell indices like Hb, MCV, MCH, MCHC and HCT were significantly decreased and RDW was significantly increased when compared with the euthyroid controls. After three months of L-thyroxine treatment, haemoglobin, RBC count, HCT, MCV, MCH, MCHC showed significant increase and there was a decrease in RDW which was statistically significant.*

***Conclusion:** Thyroid dysfunction induces varied effects on haematological parameters. In case of patients with unknown haematological dysfunction, they must be evaluated for thyroid hormones.*

KEYWORDS : Primary Hypothyroidism, Blood cell counts, Blood cell indices, L-Thyroxine

Introduction:

Thyroid gland is the largest and important endocrine gland in the human body. It is located on the anterior side of the neck, right below the larynx. Hypothyroidism is a clinical syndrome characterized by the deficiency of thyroid hormones in the target tissue, leading to generalized slowing of all metabolic processes. The thyroid gland synthesizes and releases T_3 and T_4.Biologically active hormones T_3 and T_4 play a significant role in growth, development and function of all major tissues[1]. Thyroid hormones regulate the basal metabolic rate of all the cells in the body[2]. These hormones have critical roles in early brain development, somatic growth, bone maturation, protein synthesis and regulating production of red blood cells. All these functions are regulated by attachment of the active form of the thyroid hormone T_3 to specific members of the nuclear receptors family. Hormonal output from the thyroid is mediated by thyroid stimulating hormone (TSH / thyrotropin) secreted by anterior pituitary. Secretion of thyrotropin is mediated by thyrotropin - releasing hormone (TRH) secreted by the hypothalamus. Other effects of thyroid hormones include involvement in haemoglobin production in adult and maturation of Hb in foetus [3-5]. Thyroid hormone synthesis and secretion is regulated by the negative feedback system that involves the hypothalamus, pituitary and the thyroid gland [6]. The incidence of overt hypothyroidism has been estimated to be 4.1 cases per 1000 women per year and 0.6 cases per 1000 men per year[7]. The prevalence has been reported to be approximately 1-2% in women and 0.1% in men in large population studies [8-10]. Incidence of hypothyroidism depends on different environmental and various geographic factors which include dietary iodine deficiency, genetic variation in the population.

Hypothyroidism is the most common hormonal deficiency, the diagnosis can be made quickly, confirmed and the treatment is straight forward with excellent prognosis. Thyroid hormone deficiency affects virtually every tissue in the body. Thyroid hormones often have important effect on erythropoiesis. They enhance erythropoiesis through hyper proliferation of immature erythroid progenitors and increase secretion of erythropoietin (EPO) by inducing erythropoietin gene expression. Generally it seems that hypothyroidism causes hypoplasia in all myeloid cell lineages. With regard to lymphocytes, T_3 is as a precursor substance for normal B cell formation in bone marrow through its mediation of pro-B cell proliferation. Therefore, thyroid disorders can induce different effects on various blood cell lineages [11]. Hypothyroidism can cause various forms of anaemia (normochromic-normocytic, hypochromic-microcytic or macrocytic) through reducing the oxygen metabolism. Patients with hypothyroidism have a decreased erythrocyte mass due to reduction of plasma volume and may undetectable by routine measurement such as haemoglobin concentration [12-14]. Alteration in other haematological parameters such as haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV) ,mean corpuscular haemoglobin (MCH), white blood cell (WBC) count and platelet count is associated with thyroid dysfunction [14], but all changes return to normal if an euthyroid (normal) state is obtained. Pancytopenia is a rare side effect whose cause is not well understood. Immunological mechanisms have been offered for decline of the life span of erythrocytes and platelets [15]. Since there is high prevalence of thyroid dysfunction in Indian population, the present study was undertaken to evaluate the effect of thyroid hormone deficiency on red blood cells and red blood cell indices in Shivamogga population.

Materials and methods:

With the approval of the Institutional ethics committee and the informed consent of the participants, a total number of 100 Primary hypothyroid patients of ages 30-65 years were chosen for the study. Primary hypothyroid patients were treated and after 3 months of therapy, they were evaluated again for the haematological parameters.

Exclusion criteria: Patients with cardiovascular, cerebrovascular and neurological diseases, uncontrolled hypertension, Diabetes mellitus, chronic renal failure and pregnant females were excluded from the study. All female patients were asked about their menstrual period duration, frequency, and amount of bleeding. Patients with a menstrual period lasting more than 5 days or more than usual amount of bleeding were excluded from study.

Sample collection: About 5-6 ml of venous blood was collected in EDTA Vacutainer [BD Biosciences] from antecubital vein from each patient. EDTA anti coagulated blood samples were processed in Haematology Analyser (cell counter) Erba –Sysmax (XP-100). CBC and Hemogram comprised of RBC count, White blood cells/ Total leukocyte count (TLC), Differential leukocyte count (DLC) which included lymphocyte % neutrophils % and mixed pool % , platelet (PLT) count, Hb concentration, MCV, MCH, MCHC, HCT/PCV and RDW.

Estimation of thyroid profile was done by Lilac kit by using a Chemiluminescence method. The following three parametes were estimated under thyroid profile.

1. Tri-iodo-L-thyronine (T_3)
2. Tetra-iodo-L-thyronine (T_4)
3. Thyroid stimulating hormone (TSH).

Diagnosis of Primary hypothyroidism was established based on clinical signs and symptoms and the T_3, T_4 and TSH estimations.

Statistical Analysis:
Data obtained was entered into Microsoft Excel sheet and statistical analysis was performed by SPSS software. Results were reported as mean ± standard deviation (SD) for quantitative variables and percentages for categorical variables. Statistical Independent T test was used to evaluate the significance of differences between two groups. P- Value of <0.05 was considered as statistically significant.

Results:
Table - 1 shows demographic profile of the study groups. The mean age & number of the patients in Euthyroid pool and Primary hypothyroid pool was shown in **Table-1.**

Table-2 shows the Thyroid profile of the study groups**.** Mean ±SD values of TSH, T_3, & T_4 in euthyroid pool were 3.267±1.407 µ IU/ml, 0.993±0.187 ng/dl and 8.873±2.021 µg/dl respectively. Primary hypothyroid patients were diagnosed based on clinical signs and symptoms, raised TSH level and lower T_3 & T_4 levels. Mean ±SD value of TSH, T_3 & T_4 in primary hypothyroid patients was 54.808±7.090 µ IU/ml, 0.282±0.091 ng/dl and 2.433±0.683 µg/dl respectively. After 3 months of therapy with L-thyroxine, mean ±SD values of TSH, T_3 & T_4 were 28.335±6.060 µ IU/ml, 0.565±0.063 ng/dl and 4.447±0.538 µg/dl respectively.

Table-3 shows the complete Hemogram of the study groups**.** In untreated primary hypothyroid patients, the Mean ±SD values of Blood cell counts were RBC (3.828±0.330 x10^6 cells/ cumm), WBC which included Total leukocyte count (8.429±1.820 x10^3 cells/cumm), & Differential leukocyte count like lymphocyte (33.68±8.472) % , neutrophils (61.74±8.665)%, mixed pool (4.59±1.854)% and Platelets (PLT- 2.64±6.812 x 10^5cells/ cumm) and Mean ±SD values of blood cell indices were Hb (9.314±1.451)gm%, MCV (72.923±4.600)fl, MCH (24.342±1.600) pg, MCHC (30.153±0.528)%, HCT/PCV (28.087±4.335)% and RDW (16.826±1.258)% respectively. There was a statistically significant decrease in RBC Count and blood cell indices like Hb, MCV, MCH, MCHC, HCT/PCV and increase in RDW. Patients treated with L- thyroxine therapy show correction in these erythrocyte abnormalities. As compared with patients with the euthyroid status, the RDW values showed statistically highly significant difference.

Table-4 shows the comparison of the haematological parameters and thyroid hormone levels in Untreated & treated Primary hypothyroid patients with healthy Euthyroid controls with respect to T_3, T_4 and TSH and the data was expressed as mean ± SD. The value of P<0.05, denotes that the results were statistically significant. There was no significant difference in WBC and platelet counts between euthyroid and Primary hypothyroid patients.

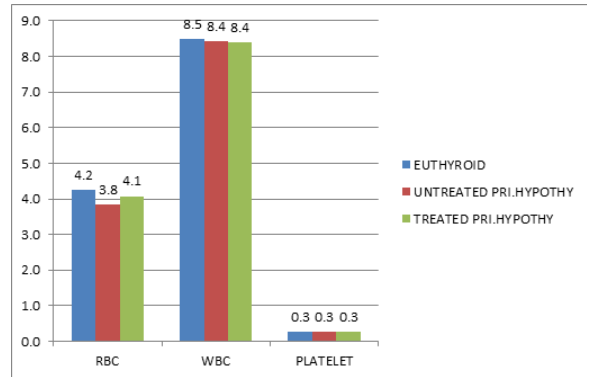

Fig 1: Blood cell counts in Euthyroid, untreated primary hypothyroid & treated primary hypothyroid. (RBC Count x 106, WBC Count x 103 and Platelet count x 105 cells/ cumm) - Bar diagram

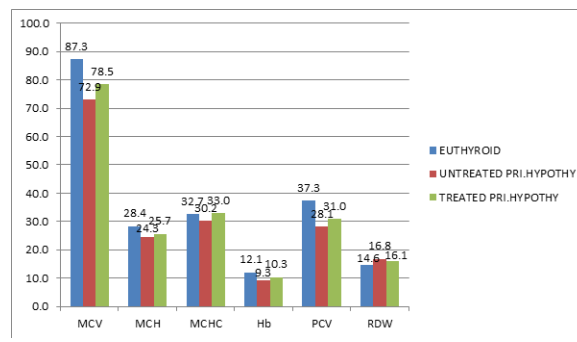

Fig 2: Red blood cell indices in Euthyroid, untreated primary hypothyroid & treated primary hypothyroid. (MCV in fl, MCH in pg, MCHC in %, Hb in gm%, HCT/PCV in % and RDW in %) - Bar diagrams

Discussion:
Although frequency of hypothyroidism varies between countries, it is a common disease. There is metabolic deceleration in hypothyroidism. According to the data of WHO (World Health Organization), anaemia is an important public health problem. In order to carry out the treatment of the patient with anaemia correctly, it is necessary to determine etiological causes. The adverse effect of hypothyroidism on the haematological system is the development of anaemia. In our study, 74% of hypothyroid patients were females. According to the data of WHO, anaemia prevalence is 24.8% throughout the world and it is seen more frequently in underdeveloped countries[16]. Thyroid hormones have crucial effect on erythropoiesis by induction of erythropoietin secretion and proliferation of erythroid progenitors [13, 17]. Thyroid hormones increase the secretion of erythropoietin (EPO) by inducing erythropoietin gene expression. Thyroid hormones augment repletion of hypoxia inducible factor-1 (HIF-1) and then induce growth of erythroid colonies (BFU-E, CFU-E) [18]. These hormones also intensify 2, 3 DPG compactness which enhances the delivery of oxygen to the tissues. Hypothyroidism causes hypoplasia in all myeloid cell lineages. Thyroid diseases are frequently associated with erythrocyte abnormalities[19]. Anaemia of hypothyroidism has been ascribed to a physiological compensation for the diminished need of tissues for oxygen. The low plasma erythropoietin levels found in hypothyroid anaemia is in accordance with this hypothesis[20].Directly or indirectly, stimulation of erythroid colony development by thyroid hormones, inhibition of the later in its absence, reduction in the oxygen distribution to tissues and diminution of erythropoietin level in the absence of thyroid hormones causes normocytic anaemia and this type of anaemia forms the most frequent type of anaemia in hypothyroid patients[21, 22]. The determination made by Christ-Crain and colleagues indicated that erythropoietin values were increased as a result of L-thyroxine treatment in women with sub clinical hypothyroidism[23]. Treatment of subclinical hypothyroidism with L-thyroxine in patients with anaemia has beneficial effect on blood count, white blood cell differential count, reticulocyte effect and blood cell indices [24]. The greater percentage of Anaemia in women with hypothyroidism may be linked to menorrhagia, as is proposed previously by Kosenli et al [25]. In this study,

that entire group with menorrhagia was excluded. But, a significant high incidence of anaemia was observed in this study, thus necessitating the need to further evaluate other causes of anaemia in hypothyroid patients. It has also been shown that in concurrent hypocoagulopathies, hypothyroidism is associated with increased risk of bleeding and haematological parameters, including MCH, MCV, MCHC, HCT and Hb significantly improve in patients with significant response to L-thyroxine [26]. Further studies need to be done in this area. In the present study also, there was decrease in RBC count, blood cell indices like Hb, MCH, MCV, MCHC and HCT. There was increase in RDW. The cause of the increase in size of the red cells and of the minor degree of anisocytosis in uncomplicated hypothyroidism is unknown [27]. Although no definitive mechanism(s) can be suggested to explain the larger prevalence of increased RDW in patients with thyroid dysfunction, results of the retrospective cross sectional analysis study suggest that abnormal levels of thyroid hormones might substantially influence the size variability of circulating RBCs [28]. After 3 months of therapy with L-thyroxine, there was improvement in haematological parameters which was statistically significant. Red cell diameter width was decreased.

Conclusion:

This study showed that, hypothyroidism is highly associated with anaemia, especially in women. Thyroid dysfunctions have a direct effect on most red blood cell indices and proper treatment of hypothyroidism can obviate treatment of concurrent anaemia. Further elaborate study is required to investigate the major endocrinal disorder of vulnerable population with its role in iron homeostasis of the body and all types of anaemia. We suggest that people with thyroid disorder should have routine screening of haematological, biochemical and hormonal profile assay and simultaneously proper management of this metabolic disease should be provided based on American endocrinologist guidelines. Hence a multisystem approach is required to treat patients suffering from hypothyroidism.

Table-1: Demographic profile of the subjects

Groups	Healthy controls / Euthyroid		Primary hypothyroid	
Sex	M	F	M	F
Number (n %)	30	70	26	74
Mean age (Yrs)	53.43	49.33	52.54	50.08

Table-2: Thyroid profile of the study groups: Mean ± SD

Parameter	Healthy controls / Euthyroid [Mean (SD)]	Untreated primary hypothyroid	Treated primary hypothyroid
TSH (µ IU/ml)	3.267±1.407	54.808±7.090	28.335±6.060
Total T3 (ng/dl)	0.993±0.187	0.282±0.091	0.565±0.063
Total T4 (µg/dl)	8.873±2.021	2.433±0.683	4.447±0.538

Table-3: Complete Hemogram of the study groups: Mean ± SD

Parameter	Healthy controls/ Euthyroid [Mean (SD)]	Untreated Primary hypothyroid	Treated Primary Hypothyroid
Hb (gm%)	12.083±1.451	9.314±1.451	10.347±0.917
Total RBC count (10^6 cells/cumm)	4.249±0.367	3.828±0.330	4.077±0.179
WBC Count / TC (10^3 cells/ cumm)	8.483±1.879	8.429±1.820	8.381±1.834
Neutrophils (%)	62.41±12.360	61.74±8.665	61.70±8.294
Lymphocytes (%)	32.31±11.284	33.68±8.472	33.86±8.336
Mixed pool (%)	5.30±2.584	4.59±1.854	4.55±1.872
Platelet count (10^5cells/ cumm)	2.67±6.532	2.64±6.812	2.65±6.619
PCV/Hematocrit (%)	37.254±4.486	28.087±4.335	31.049±3.938
MCV (fl)	87.267±4.262	72.923±4.600	78.471±2.708
MCH (pg)	28.379±1.613	24.342±1.600	25.674±1.0464
MCHC (%)	32.699±0.708	30.153±0.528	33.007±0.364
RDW (%)	14.563±1.604	16.826±1.258	16.087±0.753

***. The mean difference is significant at the 0.05 level.**

Table-4: Comparison of the haematological parameters and thyroid hormone levels in Untreated & treated primary hypothyroid patients with healthy Euthyroid controls.

Parameter	Healthy controls/ Euthyroid	Hypothyroid untreated	Hypothyroid treated	untreated primary Hypothyroid Vs Treated primary hypothyroid
TSH (µ IU/ml)	3.267±1.407	54.808±7.090 P<0.001, H.S*	28.335±6.060 P<0.001,H.S*	P<0.001, H.S*
Total T3 (ng/dl)	0.993±0.187	0.282±0.091 P<0.001, H.S*	0.565±0.063 P<0.001,H.S*	P<0.001, H.S*
Total T4 (µg/dl)	8.873±2.021	2.433±0.683 P<0.001, H.S*	4.447±0.538 P<0.001,H.S*	P<0.001, H.S*
Hb (gm%)	12.083±1.451	9.314±1.451 P<0.001, H.S*	10.347±0.917 P<0.001, H.S*	P<0.001, H.S*
Total RBC count (10^6 cells/cumm)	4.249±0.367	3.828±0.330 P<0.001, H.S*	4.077±0.179 P<0.001, H.S*	P<0.001, H.S*
WBC Count/TC(10^3 cells/cumm)	8.483±1.879	8.429±1.820 P=0.837, N.S	8.381±1.834 P=0.698, N.S	P=0.853, N.S
Neutrophils (%)	62.41±12.360	61.74±8.665 P=0.658, N.S	61.70±8.294 P=0.634, N.S	P=0.973, N.S
Lymphocytes (%)	32.31±11.284	33.68±8.472 P=0.333, N.S	33.86±8.336 P=0.271, N.S	P=0.880, N.S
Mixed pool (%)	5.30±2.584	4.59±1.854 P=0.027, Sig	4.55±1.872 P=0.020, Sig	P=0.879, N.S
Platelet (10^5 cells/cumm)	2.67±6.532	2.64±6.812 P=0.726, N.S	2.65±6.619 P=0.788, N.S	P=0.932, N.S
PCV/Hematocrit (%)	37.254±4.486	28.087±4.335 P<0.001, H.S*	31.049±3.938 P<0.001, H.S*	P<0.001, H.S*
MCV (fl)	87.267±4.262	72.923±4.600 P<0.001, H.S*	78.471±2.708 P<0.001, H.S*	P<0.001, H.S*
MCH (pg)	28.379±1.613	24.342±1.600 P<0.001, H.S*	25.674±1.046 P<0.001, H.S*	P<0.001, H.S*
MCHC (%)	32.699±0.708	30.153±0.528 P<0.001, H.S*	33.007±0.364 P<0.001, H.S*	P<0.001, H.S*
RDW (%)	14.563±1.604	16.826±1.258 P<0.001, H.S*	16.087±0.753 P<0.001, H.S*	P<0.001, H.S*

***. The mean difference is significant at the 0.05 level.**

References:

1. Boelart K, Franklyn JA. Thyroid hormone in health and disease. J Endocrinol 2005;187:1-15.
2. Dr. Ajay Kumar N, Dr. Shanthi M, Dr. Parameshwari R. The effect of L-thyroxine on metabolic parameters in newly diagnosed primary hypothyroidism. International Journal of Pharmaceutical Science Invention 2013;2(8):14-18.
3. Ahmed Ahmed OM, El-Gareib AW, El-Bakry AM, Abd El-Tawab SM, Ahmed RG. Thyroid hormones states and brain development interactions. Int J Dev Neurosci 2008; 26(2):147-209.
4. Saranac L, Zivanovic S, Bjelakovic B, Stamenkovic H, Novak M, Kamenov B. Why is the thyroid so prone to autoimmune disease? Horm Res Paediatr.2011; 75(3):157-65.
5. Koibuchi N, Chin WW. Thyroid hormone action and brain development. Trends Endocrinol Metab.2000; 11(4):123-8.
6. Shupnik MA, Ridgway EC, Chin WW. Molecular biology of thyrotropin, Endocr Rev 1989;10:459-75.
7. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty- year follow-up of the Whickham Survey. Clin Endocrinol 1995; 43:55-68.
8. Bagchi N, Brown TR, Parish RF. Thyroid dysfunction in adults over age 55 years. A study in an urban U.S. community. Arch Intern Med 1990;150:785-7.
9. Sawin CT, Chopra D, Azizi F, Mannix JE, Bacharach P. The aging thyroid. Increased prevalence of elevated serum thyrotropin levels in the elderly. JAMA 1979; 242:247-50.
10. Kochupillai N, Mehta M. Iodine deficiency disorders and their prevention in India. Rev Endocr Metab Disord 2008;9: 237-244.
11. Das KC, Mukherjee M, Sarkar TK, Dash RJ, Rastogi GK. Erythropoiesis and erythropoietin in hypo- and hyperthyroidism. J Clin Endocrinol Metab 1975; 40(2):211-220.
12. Drews RE. Critical issues in haematology: anaemia, thrombocytopenia, coagulopathy, and blood product transfusions in critically ill patients. Clin Chest Med 2003; 24(4):607-22.
13. Golde DW, Bersch N, Chopra IJ, Cline MJ. Thyroid hormones stimulate erythropoiesis in vitro. Br J Haematol. 1977; 37(2):173-7.
14. Kawa MP, Grymuła K, Paczkowska E, Baśkiewicz- Masiuk M, Dąbkowska E, Koziołek M, et al. Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. Eur J Endocrinol. 2010; 162(2):295-305.
15. Lima CS, ZantutWittmann DE, Castro V, Tambascia MA, Lorand-Metze I, Saad ST, et al. Pancytopenia in untreated patients with Graves' disease. Thyroid 2006; 16 (4):403-9.
16. Benoist B, McLean E, Egli I, Cogswell M (2008) Worldwide prevalence of anaemia 1993-2005; Global database on anaemia. WHO Geneva: 1-2.
17. Fujita H. Fine structure of the thyroid gland. Int Rev Cytol.1975; 40:197-280.
18. Dorgalaleh A, Mahmoodi M, Varmaghani B, Kiani node F et al. Effect of thyroid dysfunctions on Blood cell count and Red Blood cell Indices. Iranian Journal of Paediatric Haematology Oncology; 3 (2):73-77..
19. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol Metab. 2011; 15: S78–S81
20. Bashir H, Bhat MH, Farooq R, Majid S, Sheikh S, Rabia. Comparison of haematological parameters in untreated and treated subclinical hypothyroidism and primary hypothyroidism patients. Medical Journal of Islamic Republic of Iran. 2012; 26:172-178.
21. Kuhr T, Hala K, Dietrich H, Herold M, Wick G (1994) Genetically determined target organ susceptibility in the pathogenesis of spontaneous autoimmune thyroiditis: aberrant expression of MHC-class II antigens and the possible role of virus. J Autoimmune 7: 13-25.
22. Erdogan M, Kosenli A, Sencer G, Kulaksizoglu M. Characteristics of anaemia in subclinical and overt hypothyroid patients. Endocrine Journal 2011; 59(3):213-220.
23. Christ-Crain M, Meier C, Huber P, Zulewski H, Staub JJ, Müller B (2003) Effect of restoration of euthyroidism on peripheral blood cells and erythropoietin in women with subclinical hypothyroidism. Hormones (Athens) 2:237-242.
24. Chandel et al. Impact of subclinical hypothyroidism on iron status and haematological parameters. Annals of Pathology and Laboratory Medicine, Vol.02, No.01, Jan-Mar 2015.
25. Kosenli A, Erdogan M, Ganidagli S, Kulaksizoglu M, Solmaz S, Kosenli O, Unsal C, Canataroglu A. Anaemia frequency and etiology in Primary hypothyroidism. Endor Abstr. 2009;20;140.
26. Kazemi-Jahromi M, Shahriari- Ahmadi A, Seyed-Hosein S, Soraya D, Esmat A, Seyed Farshad A. The association between Hypothyroidism and anaemia: a Clinical study. International Journal of Haematology Oncology and Stem Cell Research. July 2010; 4(3):6-9.
27. Sahin M, Toprak SK and Altintas ND. Should Women with Abnormal Serum Thyroid Stimulating Hormone Undergo Screening for Anaemia? Arch Pathol Lab Med 2009; 133(8): 1188-9.
28. Montagnana M, Lippi G, Targher G, Salvagno GL and Guidi GC. The red blood cell distribution width is associated with serum levels of thyroid stimulating hormone in the general population. Int J Lab Hematol 2009;31:581-2.