Recent Advances in Pathology & Laboratory Medicine
Volume 4, Issue 4 - 2018, Pg. No. 4-9
Peer Reviewed & Open Access Journal

Research Article

Glycosylated Hemoglobin among Non-diabetic Patients Diagnosed as Benign Thyroid Lesions on Cytology: A Cross Sectional Study from a Tertiary Care Centre in India

Sujata Raychaudhuri[1], Reetika Menia[2], Mitasha Singh[3], Mukta Pujani[4], Nimisha Sharma[5], Teena Gupta[6], Harnam Kaur[7]

[1,4]Associate Professor, [2]Tutor, [5]Assistant Professor, Department of Pathology, ESIC Medical College and Hospital Faridabad, Haryana.
[3]Assistant Professor, Department of Community Medicine, ESIC Medical College and Hospital Faridabad, Haryana.
[6]M.B.B.S Student, ESIC Medical College and Hospital Faridabad, Haryana.
[7]Professor and Head, Department of Biochemistry, ESIC Medical College and Hospital Faridabad, Haryana.
DOI: https://doi.org/10.24321/2454.8642.201814

Abstract

Background: Hypothyroidism and diabetes usually coexist and are the most common endocrine disorders seen in India (1). Glycosylated Hb (HbA1c) is used for assessment of glycemia and American Diabetic Association (ADA) has recommended its use in diabetes and prediabetes (2). A value between 5.7% and 6.5% represents prediabetes while a value ≥6.5% is considered as diabetes mellitus (3). Glycosylated hemoglobin is a fraction of hemoglobin that undergoes non-enzymatic glycation over the circulatory life span of erythrocytes (4). Several studies have shown glycosylated Hb varies in different conditions like hemoglobinopathies, pregnancy and chronic kidney disease (5).

Thyroid hormone plays an important role in glucose homeostasis (6). TSH regulates hematopoiesis in bone marrow (7). Hypothyroidism depresses the marrow which causes decreased erythrocyte production which alters the life span of erythrocytes. Altered life span causes spurious elevation of HbA1C (8, 9, 10). Hence, glycosylated Hb not only depends on glycemia but also on life span of RBC (11). Conditions which effect erythrocyte turnover or survival lead to falsely high or low Hb A1C levels (12). RBC turnover is increased in thyrotoxic states whereas hypothyroidism has the opposite effect (3).

In the present study, we hypothesise that glycosylated hemoglobin shows variation in individuals with altered thyroid status. It also aim to establish if a correlation exits between fasting plasma glucose level and hemoglobin with glycosylated hemoglobin in patients with altered thyroid status.

Aims and Objectives: To find a correlation between thyroid profile and glycosylated Hb in non-diabetic patients who have been diagnosed on cytology as benign thyroid lesions and Compare the fasting blood glucose and hemoglobin with glycosylated Hb in these patients.

Material and Methods: A cross sectional study on 50 cases cytologically diagnosed as benign thyroid lesions in the Department of Pathology in ESIC Medical College and Hospital Faridabad were included in the study with consent of ethical committee.

Corresponding Author: Dr. Reetika Menia, Department of Pathology, ESIC Medical College and Hospital, NIT Faridabad, Haryana.
E-mail Id: drreet830@gmail.com
Orcid Id: https://orcid.org/0000-0002-9864-2081
How to cite this article: Raychaudhuri S, Menia R, Singh M et al. Glycosylated Hemoglobin among Non-diabetic Patients Diagnosed as Benign Thyroid Lesions on Cytology: A Cross Sectional Study from a Tertiary Care Centre in India. *Rec Adv Path Lab Med* 2018; 4(4): 4-9.

Data Analysis: Pearson's coefficient was applied to test the association between variables. The significance level was set at 5%.

Results: Out of 50 patients (n=25) 50% were hypothyroid, (n=13) 26% were hyperthyroid and (n=12) 24% were euthyroid and (n=22) 88% hypothyroid patients presented with HbA1C >6.5% and were labeled as Diabetic, (n=3) 12% hypothyroid patients were labelled as prediabetic and none was nondiabetic. Most of the euthyroid (n=11) 92% and all of the hyperthyroid patients (n=13) 100% had HbA1C in the nondiabetic range of <5.7%. Only one euthyroid patient (8%) had HbA1C in the prediabetic range. It was observed that microcytic hypochromic anemia was commoner in hypothyroid patients with HbA1C in diabetic range (HbA1C>6.5%). The correlation of HbA1C with TSH, Hb and MCH of these patients showed statistical significance (p <0.001). Relation of FBS with HbA1C was not significant.

Conclusion: The study suggests that physicians dealing with patients having altered thyroid status should interpret glycosylated hemoglobin with caution before labelling them as diabetic (HbAIc >6.5%) or prediabetic (HbAIc between 5.7 to 6.5%).

Keywords: Anemia, Benign Thyroid, FBS, Glycosylated Hemoglobin

Introduction

Hypothyroidism and diabetes usually coexist and are the most common endocrine disorders seen in India.[1] Glycosylated Hb (HbA1C) is used for assessment of glycemia and American Diabetic Association (ADA) has recommended its use in diabetes and prediabetes.[2] A value between 5.7% and 6.5% represents prediabetes while a value ≥6.5% is considered as diabetes mellitus.[3] Glycosylated hemoglobin is a fraction of hemoglobin that undergoes non-enzymatic glycation over the circulatory life span of erythrocytes.[4] Several studies have shown glycosylated Hb varies in different conditions like hemoglobinopathies, pregnancy and chronic kidney disease.[5]

Thyroid hormone plays an important role in glucose homeostasis.[6] TSH regulates hematopoiesis in bone marrow.[7] Hypothyroidism depresses the marrow which causes decreased erythrocyte production which alters the life span of erythrocytes. Altered life span causes spurious elevation of HbA1C.[8-10] Hence, glycosylated Hb not only depends on glycemia but also on life span of RBC.[11] Conditions which effect erythrocyte turnover or survival lead to falsely high or low Hb A1C levels.[12] RBC turnover is increased in thyrotoxic states whereas hypothyroidism has the opposite effect.[13]

In the present study, we hypothesize that glycosylated hemoglobin shows variation in individuals with altered thyroid status. It also aims to establish if a correlation exits between fasting plasma glucose level and hemoglobin with glycosylated hemoglobin in patients with altered thyroid status.

Aims and Objectives

- To find a correlation between thyroid profile and glycosylated Hb in non-diabetic patients who have been diagnosed on cytology as benign thyroid lesions
- Compare the fasting blood glucose and hemoglobin with glycosylated Hb in these patients.

Material and Methods

A cross sectional study on 50 cases presenting with benign thyroid lesions on cytology in the Department of Pathology was conducted with prior approval of Institutional Ethical Committee. Informed consent from the patients was taken. These patients were investigated for Thyroid profile, fasting blood glucose, CBC and glycosylated Hb (Table 1). Venous blood samples were collected using vacutainer in EDTA, plain and glucose vials. Complete Blood Count (CBC) was analyzed using fully automated hematology analyzer XN 1000. ELISA in ERBA analyzer used for thyroid hormones Triiodothyronine, Thyroxine and Thyroid Stimulating Hormone (T3, T4 and TSH). Randox Daytona was used for glycosylated Hb estimation (Table 1).

Sample

The Department of Pathology receives on an average thyroid sample of 25 patients in a month. Taking data collection period as 2 months (July and August 2018) population size was calculated to be 50.

Inclusion Criteria

All patients with benign thyroid disorder and fasting blood glucose levels with in normal limits were included for the study.

Exclusion Criteria

All those patients with previous history of thyroid disorders including gestational thyroid disorders and those on thyroid medication or those who have undergone thyroidectomy or have history of radiation exposure or chronic renal failure will be excluded from the study.

Data Collection

The profile of the study participants who met the criteria and consent to participate was recorded in a semi-structured pre-designed questionnaire. The socio demographic profile of the patient, relevant clinical history and lab findings were also recorded in the study tool. The data collection was done

ISSN: 2454-8642
DOI: https://doi.org/10.24321/2454.8642.201814

in lab when patient visited for cytology sample. This was followed by blood sampling using universal precautions.

Data Analysis

Data analysis was done using SOFA STATS version 1.4.6. Categorical variables were summarized as frequencies (percentage), while the continuous variables were summarized as median (interquartile range).

The comparison of the proportions was done using the test of proportions. Pearson's coefficient of correlation was calculated to determine the correlation between two variables. P value less than 0.05 was considered significant.

Microcytic hypochromic anemia was defined as microcytic hypochromic picture on peripheral smear with low Hb levels (<12g% in males, <11g% in female) and with MCV <76 fL and MCH<27 pg/cell. Normocytic normochromic anemia was defined as low Hb levels but with normocytic normochromic red cell indices along with peripheral smear picture. The red blood cell count (RBC) cutoffs were RBC <4.5million/ μL for males and RBC <4.2 million/uL for females. The elevated levels of the thyroid hormones T3, T4, and TSH were based on the following cutoffs, T3 >4.2 pg/dL, T4 >2.3 g/dL and TSH >7.0 mIU/L.

All the patients were non-diabetic (Fasting Plasma Glucose <100 mg/dl) and HbA1C was performed. HbA1C >6.5% was taken as Diabetic, values between 6.5% and 5.7% were considered as prediabetic and values <5.7% were labeled as nondiabetic.

Results

In our study, 50 cases with cytological diagnosis of benign thyroid lesions (TBSRTC Cat II Bethesda 2014) were studied and they were sampled for Thyroid profile, HbA1C and other hematological parameters. The age distribution of patients was in the range of 20 to 55 (Table 2) and the mean age was 41.95 years. The male to female ratio was 1:25. Out of 50 patients, (n=25) 50% were hypothyroid, (n=13) 26% patients were labeled as hyperthyroid and (n=12) 24% as euthyroid. It was further found that (n=22) 88% hypothyroid patients presented with HbA1C >6.5% and were labeled as Diabetic, (n=3) 12% hypothyroid patients had HbA1C in the prediabetic range and none in the nondiabetic range (Figure 1). Most of the euthyroid (n=11) 92% and all of the hyperthyroid patients (n=13) 100% had HbA1C in the nondiabetic range of <5.7%. Only one of the euthyroid patient (8%) had HbA1C in the prediabetic range (Fig. 1, table 3).

It was seen that out of 25 hypothyroid patients, 72% (n=18) patients had microcytic hypochromic anemia, 8% (n=2) had normocytic normochromic anemia and 20% (n=5) were non-anemic. Out of 13 hyperthyroid patients, 16% (n=2) had microcytic hypochromic anemia, 23% (n=3) had normocytic normochromic anemia and 61% (n=8) were non-anemic. It was also noted that none of the euthyroid patients had microcytic hypochromic anemia, 34% (n=4) had normocytic normochromic anemia and 66% (n=8) were non-anemic (Fig 2, 3 and table 4).

Table 1.Patient characteristics

	Microcytic hypochromic	Normocytic normochromic	Non anemic
Hemo-globin (gm /dl)	9.28±2.5	9.5±0.56	13.1±1.9
MCV(FL)	56.8 ±7.2	79.2±9.28	81 ±4.5
MCH (pg)	17 ±4.9	32.1±6.9	50.5 ±4.2
Fasting blood sugar (mg/dl)	85.8 ±9.1	88.2±5.4	86 ±7
HbA1C (%)	6.8±0.57	6.2±0.51	5 ±0.76
TSH (mIU/L)	33.2 ±10.9	29.6 ±10.4	35.5 ±15.5

Table 2.Age distribution with thyroid levels

Age (years)	Hypothyroid	Hyperthyroid	Euthyroid
20-25	6%	0%	0%
26-30	0%	4%	6%
31-35	0%	2%	4%
36-40	6%	2%	2%
41-45	16%	10%	6%
46-50	6%	4%	4%
51-55	14%	6%	2%

Table 3.Relation of HbA1C with thyroid profile

	Diabetic (>6.5%)	Prediabetic (5.7 to 6.5%)	Non diabetic (<5.7%)
Hypothyroid (n=25)	22	3	0
Hyperthyroid (n=13)	0	0	13
Euthyroid (n=12)	0	1	11

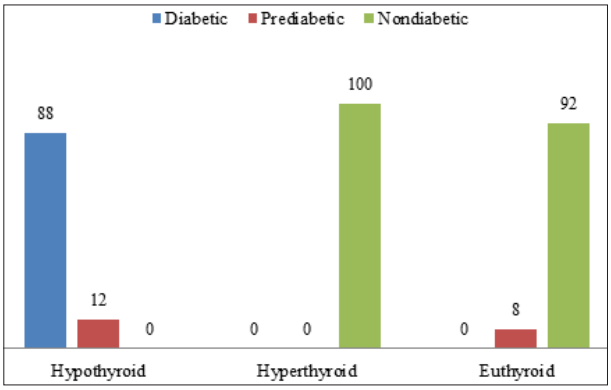

Figure 1.Relation of HbA1C with thyroid profile

ISSN: 2454-8642
DOI: https://doi.org/10.24321/2454.8642.201814

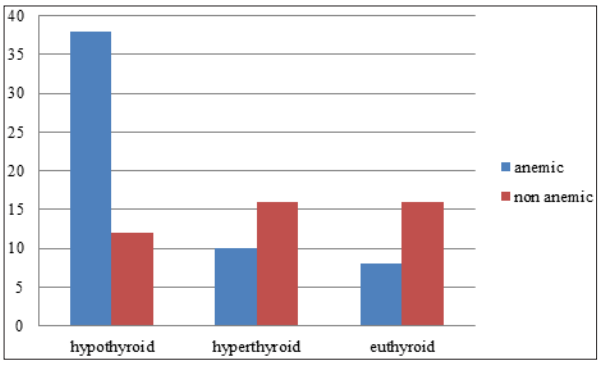

Figure 2.Relation of thyroid profile with anemia

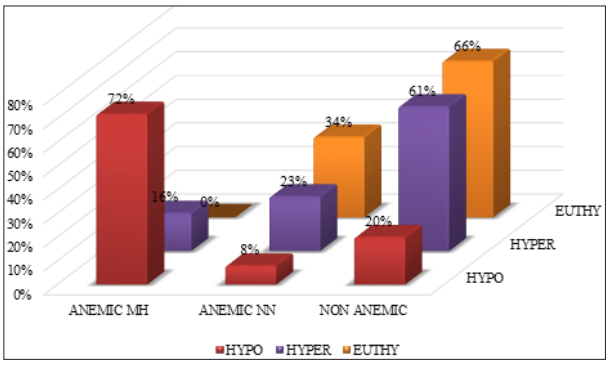

Figure 3.Relation of thyroid profile with type of anemia

Table 4.Relation of thyroid profile with type of anemia

Anemia	Hypothyroid (n=25)	Hyperthyroid (n=13)	Euthyroid (n=12)
Microcytic hypochromic	72% (n=18)	16% (n=2)	0%
Normocytic normochromic	08% (n=2)	23% (n=3)	34% (n=4)
Non-anemic	20% (n=5)	61% (n=8)	66% (n=8)

Table 5.Correlation of thyroid status with glycosylated Hb and anemia

		Microcytic Hypochromic	Normocytic Normochromic	Non Anemic
Hypothyroid	Diabetic	16	0	06
	Pre Diabetic	0	0	0
	Non Diabetic	0	0	0
Hyperthyroid	Diabetic	0	0	0
	Pre Diabetic	0	0	0
	Non Diabetic	2	3	8
Euthyroid	Diabetic	0	0	0
	Pre Diabetic	0	1	0
	Non Diabetic	0	4	8

It was also noted that out of 22 hypothyroid patients with HbA1C in diabetic range, 73% (n=16) presented with microcytic hypochromic anemia (p<0.001) whereas (n=6) 27% patients (diabetic range) did not show anemia and all the patients with HbA1C in prediabetic range (n=3) also showed microcytic hypochromic anemia (Table 5, Fig 3).

It was found that out of 12 normoglycemic hyperthyroid patients, 34% (n=4) had normocytic anemia and none of the euthyroid patients presented with microcytic hypochromic anemia. Only 23% hyperthyroid patients presented with normocytic normochromic anemia.

So, it was concluded that microcytic hypochromic anemia was commoner in hypothyroid patients with HbA1C in diabetic range (HbA1C>6.5%) (p<0.001).

Chi square test was applied to test the association of HbA1C and MCV. It was found to be significant with p<0.001.

Pearson's coefficient also showed strong correlation between TSH and HbA1C p<0.001 but the correlation of HbA1C with T3, T4 was not significant (p= 0.69, 0.21) respectively.

It was also noted that Hb level of these thyroid patients showed statistical significance with glycosylated hemoglobin (p < 0.001).

There was weak and borderline significant negative correlation found between HbA1C and MCV in microcytic hypochromic anemia, r = -0.43, p=0.06 but the relation between HbA1C and MCH, was significant (p<0.01). While in case of normocytic normochromic anemia no such correlation was found.

Discussion

In our study of 50 cases, we found that mean age of patients was 41.9 yrs. This was in accordance to a study by Mehmet MA et al. where the mean age of patients was 44.3 years.[13] The male to female ratio in our study was 1:25. A study by Iddah E et al. also showed female preponderance in their study with male to female ratio being 1:10.9.[14]

It was also found that HbA1c level was significantly higher in overt hypothyroid patients. Our findings were similar to the observations by Kim MK et al.[15] This may be due to the low RBC turnover in hypothyroid patients.[15] Our study

ISSN: 2454-8642
DOI: https://doi.org/10.24321/2454.8642.201814

showed that elevation of HbA1C was in the diabetic and prediabetic range in all hypothyroid patients and out of these 73% patients presented with microcytic hypochromic anemia. The most common cause of microcytic anemia is iron deficiency. Endocrine diseases, thalassemia and anemia of chronic diseases are also the causes. A study by Rakesh et al. suggested that the patients with microcytic hypochromic anemia due to iron deficiency had higher levels of HbA1C when compared to anemia of endocrine diseases but there was no significant correlation found between HbA1C and TSH & Plasma glucose levels and TSH in his study[16]. However, in the present study, the cause of microcytic hypochromic anemia could not be attributed to iron deficiency alone as serum iron studies were not under taken, but significant correlation was found between HbA1C and TSH ($P<0.001$).

It was also noted that 73% (n=16) hypothyroid patients with HbA1C in the diabetic range presented with microcytic hypochromic anemia ($p<0.001$), (n=3) all the patients with HbA1C in prediabetic range also showed microcytic hypochromic anemia whereas (n=5) 27% hypothyroid patients (diabetic range) did not show anemia.

It was found that two normoglycemic hyperthyroid patients had microcytic hypochromic anemia and none of the euthyroid patients presented with microcytic hypochromic anemia. An equal number of euthyroid and hyperthyroid patients (n=8) presented with normocytic normochromic anemia.

The results of our study were in concordance with the previous reports as microcytic hypochromic anemia and normocytic normochromic anemia were the only two types of anemia detected in hypothyroid patients.[17] The frequency of both types was significantly higher in hypothyroidism compared to normal thyroid group.

Hypothyroidism and diabetes are the most common coexisting endocrine disorders found in Indian population.[18] The prevalence of thyroid disease in patients with diabetes mellitus is approximately 10-15%.[19] Previous studies done in hypothyroid patients have shown that HbA1C can be elevated in non-diabetic subjects too. Hence the role of HbA1C as a marker of diabetes was questioned in such conditions especially when American Diabetes Association has endorsed it as diagnostic criteria for diabetes mellitus. Further studies that were done to evaluate the cause of these elevated HbA1C, Kim MK et al.[15], found that they were attributed to anemia associated with it. Many studies in the literature have shown that iron deficiency anemia is mostly associated with elevated HbA1C level.[5] Other conditions where iron deficiency anemia plays an important role in elevating HbA1C levels are chronic kidney diseases and pregnancy. Hypothyroidism is mainly complicated by normocytic normochromic anemia which may be early iron deficiency anemia due to nutritional deficiency or it may be secondary to hypothyroidism itself. [18,19]

There was no significant correlation found between HbA1c and erythrocyte indices in case of normocytic normochromic anemia. Previous studies have found association between red cell survival and elevated HbA1c levels.[19-21] Hence , red cell morphology alone may not completely explain the elevated HbA1C levels; rather red cell survival time gives a better explanation of it. We did not measure the erythrocyte lifespan, which was one of the limitations of our study.

In anemic patients, the concentration of glycated hemoglobin has been reported to be increased despite the shortened life span of the erythrocytes. Several mechanisms have been attributed to this increase in the levels of glycated hemoglobin in anemic patients.[20] Some studies proposed that in iron deficiency, the quaternary structure of the hemoglobin molecule may be altered, and that the glycation of the β-globin chains occurs more readily.[21] Some studies also suggest that the increase in the glycated hemoglobin levels in non-diabetic anemic patients has been mainly attributed to the decrease in the hemoglobin levels in these patients. Our study is clinically relevant because iron deficiency anemia is highly prevalent in India and the study population is predominantly females in different age groups with mean age of 41.95 years. Females are more prone to develop thyroid disorders with anemia, especially during the reproductive age and pregnancy.[17] The anemia may be attributed to insufficient dietary intake of iron, menstrual blood loss, menorrhagia and pregnancy. Iron plays role an important role in hematopoiesis and influences the HbA1c levels. So, we must correct Iron deficiency before making any diagnostic or therapeutic decision based on the HbA1c levels. HbA1c is commonly used to assess the long-term blood glucose control in the patients with diabetes mellitus, because the HbA1c value has been shown to predict the risk for the development of many of the chronic complications in diabetes.

Conclusion

This study is of significant relevance in the Indian subcontinent where iron deficiency and thyroid disorder are very common in females of reproductive age group.

The study suggests that physicians dealing with patients having altered thyroid status should interpret glycosylated hemoglobin with caution before labeling them as diabetic or prediabetic as per ADA criterion based on levels of glycosylated Hb.

The uniformity of measurement of glycosylated hemoglobin using different principles is must to make the data more comparable with other larger studies which includes people of different ethnic and socioeconomic group to make them more useful for daily references. This study can be undertaken on a larger scale and over an extended period of time in government and corporate hospitals to obtain precise results. This study was time bound which added to the limitations. Also, we could not correlate the

ISSN: 2454-8642
DOI: https://doi.org/10.24321/2454.8642.201814

erythrocyte indices with serum ferritin and iron levels to attribute the cause of microcytic hypochromic anemia to iron deficiency. The post therapy data of the patients could not be obtained, and hence could not explain the effect of thyroid hormone therapy on HbA1C levels. As the sample size was small, the statistical significance of glycosylated hemoglobin with various parameters was limited in the present study.

Acknowledgement: None

Conflict of Interest: None

References

1. Christy AL, Manjrekar P, Babu RP et al. Elevation of HbA1C in non-diabetic hypothyroid individuals: is anaemia the connecting link? - a preliminary study. *JCDR* 2013; 7(11): 2442-44.
2. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2010; 33: S62-9.
3. Bhattacharjee R, Thukral A, Chakraborty PP et al. Effects of thyroid status on glycated hemoglobin. *Indian Journal of Endocrinology and Metabolism* 2017; 21(1): 26-30.
4. Anantarapu S, Vaikkakara S, Sachan A et al. Effects of thyroid hormone replacement on glycated hemoglobin levels in non-diabetic subjects with overt hypothyroidism. *Arch Endocrinol Metab* 2015; 59(6): 495-500.
5. Nitin S, Mishra T, Tejinder S et al. Effect of iron deficiency anaemia on haemoglobin A1c levels. *Ann Lab Med* 2012; 32: 17-22.
6. Yadav P, Kaushik GG, Sharma S. Importance of screening type-II diabetics for thyroid dysfunction and dyslipidemia. *International Journal of Biochemistry and Biophysics* 2015; 3(2): 7-12.
7. Golde DW, Bersch N, Chopra IJ, et al. Thyroid hormones stimulate erythropoiesis in vitro. *The British Journal of Haematology* 1977; 37: 173-7.
8. Horton L, Coburn RJ, England JM et al. The hematology of hypothyroidism. *Q J Med* 1975; 45(177): 101-23.
9. Das KC, Mukherjee M, Sarkar TK et al. Erythropoiesis and erythropoietin in hypo and hyperthyroidism. *J Clin Endocrinol Metab* 1975; 40: 211-20.
10. Fein HG, Rivlin RS. Anaemia in thyroid diseases. *Med Clin North Am* 1975; 59: 1133-45.
11. Ram VS, Kumar G, Kumar M et al. Association of subclinical hypothyroidism and HbA1c levels in non-diabetic subjects attending rural tertiary care centre in Central India. *International Journal of Research in Medical Sciences* 2017; 5(8): 3345-9.
12. World Health Organization. Prevention and control of Iron Deficiency Anemia in women and children: Report of the UNICEF/WHO Regional Consultation February 1999. World Health Organization, Geneva. 2001.
13. Mehmet E, Aybike K, Ganidagli S et al. Characteristics of anemia in subclinical and overt hypothyroid patients. *Endocrine Journal* 2012; 59(3): 213-20.
14. Iddah MA, Macharia BN, Ng'wena AG et al. Thyroid hormones and hematological indices levels in thyroid disorders patients at Moi Teaching and Referral Hospital, Western Kenya. *ISRN Endocrinology* 2013; 1-6.
15. Kim MK, Kwon HS, Baek KH et al. Effects of thyroid hormone on A1C and glycated albumin levels in nondiabetic subjects with overt hypothyroidism. *Diabetes Care* 2010; 33(12): 2546-8.
16. Dhadhal R, Chabra RJ, Mangukiya K et al. A Study of Glycated Hemoglobin (HbA1c) in Non Diabetic Hypothyroid Population. *IJHSR* 2015; 5(3): 127-132.
17. Vanderpump M. Thyroid autoimmunity following an iodization programme. *Clin Endocrinol (Oxf)* 2011; 75: 101.
18. Hardikar PS, Joshi SM, Bhat DS et al. Spuriously high prevalence of prediabetes diagnosed by HbA(1c) in young Indians partly explained by hematological factors and iron deficiency anaemia. *Diabetes Care* 2012; 35(4): 797-802.
19. Diez JJ, Iglesias P, Burman KD. Spontaneous normalization of thyrotropin concentrations in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab* 2005, 90: 4124-7.
20. Koga M, Morita S, Saito H et al. Association of erythrocyte indices with glycated haemoglobin in premenopausal women. *Diabet Med* 2007; 24: 843-7.
21. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anaemia on the levels of haemoglobin A1c in nondiabetic patients. *Acta Haematol* 2004; 112: 126-8.

Date of Submission: 2018-09-26
Date of Acceptance: 2018-10-09