



Maternal thyroid status and its relation to Ferritin & Vitamin B12 in Saudi pregnant women

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Abstract

Background: - Thyroid dysfunction is a worldwide phenomenon in women and the prevalence increases during pregnancy, with hypothyroidism being the most common.

In most developing countries, nutritional deficiencies of nearly all essential nutrients, including iron, vitamin B12 and folic acid are common in pregnant women. Thyroid disorders and nutritional deficiencies especially of iron and vitamin B12 cause a number of maternofetal complications.

Objectives: - to assess thyroid status in pregnant Saudi women and explore its relation to ferritin and vitamin B12.

Subjects and methods: -This was a cross sectional study conducted at antenatal clinics of the Northern Area Armed Forces Hospital (NAAFH), KSA. Enrolling 254 Saudi women; 180 pregnant [classified according to gestational age into group A (1st trimester) and group B (2nd trimester)], and 74 age matched healthy non-pregnant women as control group C. After a detailed obstetrical and medical history, and clinical assessment, participants were subjected to laboratory investigations in the form of thyroid function by measuring thyroid stimulating hormone (TSH) and free thyroxin (free T4), Haemoglobin (HB) , serum ferritin (s.F) and vitamin B12 level.

Results: - TSH level was lower in pregnant than non-pregnant. Subclinical hypothyroidism (35.5%) was the most common thyroid disorder followed by overt hypothyroidism (10%) and hypothyroxinaemia (2.2%) in pregnant women. Hemoglobin and vitamin B12 levels were significantly lower in first and second trimester of pregnancy when compared to controls ($p=0.001$). Serum FT4 correlated positively with HB and ferritin, while TSH correlated negatively with HB and ferritin.

Conclusion: - High prevalence of hypothyroidism in pregnant females and its association with iron and vitamin B 12 deficiencies highlight the urgent need for thyroid status to be detected and to evaluate nutritional deficiencies in such group, so as to early treatment started promptly and to prevent the adverse effects of the disorder to both mother and foetus to achieve normal pregnancy outcome.

Keywords: thyroid, pregnancy, ferritin, vitamin B12.

1. Introduction

The prevalence of thyroid disorder in pregnancy is around 2-5%, with hypothyroidism being more common, about 6.47-14.32% [1]. During the initial phase of gestation, the fetus is solely dependent on thyroid hormones from mother until its own thyroid gland is developed. Inadequate supply of thyroid hormones at this stage can cause fetal brain damage, miscarriage or premature growth of fetus [2].

In pregnant women; the gland increases 10% in size in iodine-replete countries and by 20% – 40% in areas of iodine deficiency. Production of thyroxine (T4) and triiodothyronine (T3) increases by 50%, along with a 50% increase in the daily iodine requirement. This is due to estrogen induces a rise in serum thyroid binding globulin, while the placenta releases several thyroid stimulatory factors in excess like human chorionic gonadotropin (HCG) [3]. During the 1st trimester (HCG) induces a transient increase in (FT4) levels, which is mirrored by a lowering (TSH) concentrations. Following this period, or 2nd trimester, serum FT4 concentrations decrease of approximately 10 to 15% and serum TSH values steadily return to normal [4], while others said; serum FT3 and FT4 levels decrease gradually from the first to the last three months of pregnancy, and TSH level increases gradually during the whole pregnancy, these physiological changes may result in hypothyroidism [5].

In our clinical practice we have noticed TSH elevated rapidly along with the decrease of haemoglobin (Hb) and serum ferritin (SF) levels in pregnant women. A few clinical studies have indicated that iron deficiency affects thyroid function during pregnancy. *Yu X et al.*, found that iron deficiency (ID) is an independent risk factor for isolated hypothyroxinemia during the first trimester of pregnancy with appropriate iodine intake in China [6]. *Zimmermann MB.*, provided data suggesting that poor maternal iron status predicts higher TSH and lower FT4 during pregnancy in areas of borderline iodine deficiency [7].

Vitamin B12 maintains normal folate metabolism which is essential for cell multiplication during pregnancy. Vitamin B12 deficiency is emerging as a growing public health problem and an increasing number of studies show that deficiency is commonly seen in pregnancy [8]. Vitamin B12 status during pregnancy is critical since maternal vitamin B12 deficiency can affect the pregnancy outcome for both mother and the offspring. For women who want to get pregnant, a vitamin B12 deficiency means an

increased risk of developing intra-uterine growth retardation, preeclampsia, and preterm labor [9]. Deficiency of vitamin B12 is highly prevalent among hypothyroid patients. Vitamin B12 deficiency worsens hypothyroidism. Unfortunately, both deficiencies can go unnoticed and they can be difficult to diagnose [10].

So, the present study was aimed to assess thyroid status in pregnant women in 1st and 2nd trimester and explore its relation to ferritin and vitamin B12.

2. Subjects and Methods

2.1. Study design:

In this cross sectional study, 180 Saudi pregnant women in their 1st trimester or 2nd trimester were recruited from antenatal clinics of the Northern Area Armed Forces Hospital (NAAFH), KSA. In the period from January 2018 to April 2018. Participants were subdivided into two groups; group (A) pregnant women in the 1st trimester (1-13 week), and group (B) pregnant women in the 2nd trimester (14-27 week). Another 74 age matched healthy non-pregnant Saudi women were served as controls [Group C]. The sample size was based on the number of patients who met the inclusion criteria during the study period.

The criteria for selecting the pregnant individuals in the sample included:

- Pregnant in the 1st or 2nd trimester with a viable normal fetus.
- Coming for routine obstetric evaluation did not start any supplementation before.
- Singleton pregnancy.

Exclusion criteria for studied groups:

- Multiple pregnancies.
- History of: thyroid disease or any other chronic illness; renal, diabetic, etc.
- On any supplementation 6 months before the study.

2.2. After informed consent; all participants were subjected to:

- History taking including: socio demographic and medical information.
- Complete physical examination including abdominal ultrasound to pregnant women to

confirm gestational age and normality of pregnancy.

- At their first antenatal visit as part of routine laboratory workup, all the participants were subjected to the following laboratory investigations:
 1. Thyroid function by measuring thyroid stimulating hormone (TSH) and free thyroxin (free T4).
 2. Haemoglobin (HB) and serum ferritin (s.F).
 3. Vitamin B12 level.

Blood was collected after 8 – 10 hours of fasting by venipuncture from all participants. TSH, free T4, HB, serum ferritin and vitamin B12 measurements were performed using the chemiluminescence assay (Roche Diagnostics, Cobas 6000 analyzer e601 module, Germany). The normal range according to the manufacturer for TSH and free T4 was 0.27-4.20 μ IU/mL and 12-20 pmol/L, respectively. The detection sensitivity was 0.005 μ IU/mL for TSH and 0.3 pmol/L for FT4. The normal range for ferritin kit was 13-150 ng/mL, for HB was 12-15.5 g/dl; and for vitamin B12 was 153-710pmol/L.

2.3. Thyroid status and definitions:

According to the guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy [11]. And according to the currently available report that included normal pregnant participants from the Gulf Region [12]; the normal range for FT4 during pregnancy was (10.5 to 22.3 pmol/L). Thyroid disorders during pregnancy were classified into:

- **Hypothyroidism** was considered when TSH >2.5 μ IU/mL and the level of FT4 defined the type as either primary (FT4 <10.5 pmol/L) or subclinical (FT4 10.5 pmol/L).
- **Hyperthyroidism** was considered for TSH 0.03 μ IU/mL and/or FT4 > 22.3 pmol /L.
- **Hypothyroxinaemia** was define by the presence of low FT4 (10.5 Pmol /L) with normal serum TSH levels (2.5 μ IU/mL).
- **Euthyroid** was defined by normal TSH (2.5 TSH > 0.3 μ IU/mL) and normal FT4 (10.5 FT4 22.3 pmol/L) level.

For the non pregnant (control group);

- **Hypothyroidism** was considered when TSH >4.2 μ IU/mL and the level of FT4 defined the type as either primary (FT4 <10 pmol/L) or subclinical (FT4 10 pmol/L).
- **Hyperthyroidism** was considered for TSH 0.1 μ IU/mL and/or FT4 >23 pmol / L.
- **Hypothyroxinaemia** was define by the presence of low FT4 (10 Pmol /L) with normal serum TSH levels (4.2 μ IU/mL).
- **Euthyroid** was defined by normal TSH (4.2 TSH > 0.3 μ IU/mL) and normal FT4 (10.5 FT4 23 pmol/L) level.

3. Statistical Analysis

Software (SPSS, Version 20.0 for Windows, SPSS Inc, Chicago, IL) was used for the univariate, bivariate, and stratified analyses of the data. Qualitative variables were analyzed by constructing contingency tables with Fisher exact test (FET), when conditions for the former were not met. Analysis of variance (ANOVA test F) and kruskal-wallis test was used for multiple comparisons of quantitative variables of parametric and non-parametric type.

Correlations among variables were studied by using the Pearson coefficient (r). Differences were considered significant at P .05 and non-significant at .05.

4. Results

254 women participated in the study, 180 of them (70.8%) were pregnant. According to their gestational age they were classified into 2 groups; group A: 104(40.9%) women in the 1st trimester (1-13 week) and group B: 76(29.9%) women in the 2nd trimester (14-27 week). 74(29.2%) age matched healthy non-pregnant Saudi women were served as controls (group C). Mean age of group A was (30.6 \pm 6.6) year, mean age of group B was (28.8 \pm 6.9) and mean age of group C was (33.1 \pm 9.9) year with statistical significant difference between group C in relation to group A and B (P =0.004). As regard body mass index [BMI =weight (kg)/height (m²)], there was statistical significant difference between group C in relation to group A and B (P =0.013), table 1.

Table (1) shows the characteristics of the study subjects:

	Group A 104(40.9%)	Group B 76(29.9%)	Group C 74(29.2%)	F test	p
Age (years) Mean ±SD	30.6±6.6	28.8±6.9	33.1±9.9 ^{ab}	5.7	0.004**
BMI "kg/m ² " Mean ±SD	27.7±6.3	28.1±5.4	30.4±6.6 ^{ab}	4.45	0.013*

Data are presented as the mean ±SD.

*P value for differences between groups. ab Significance compared to group A & B.

Compared with group C; group A and group B pregnant women exhibited non significant lower TSH levels: (2.76±2.88 vs. 2.57±1.44 and 2.59±1.11 mIU/L, P =0.25). The TSH levels were similar in groups A and B pregnant women (2.57±1.44 and 2.59±1.11 mIU). Significant differences were observed in FT4 levels among the studied groups. Compared with group A; FT4 levels were significantly lower B and C (14.77±2.13 vs. 12.94±1.89 and 13.56±2.89 pmol/L, P =0.001). Compared with group C, pregnant women in groups A and B showed remarkable low vitamin B12 levels (425.46±158.85 vs. 192.60±76.2 and 165.05±74.9 pmol/L, P =0.001).

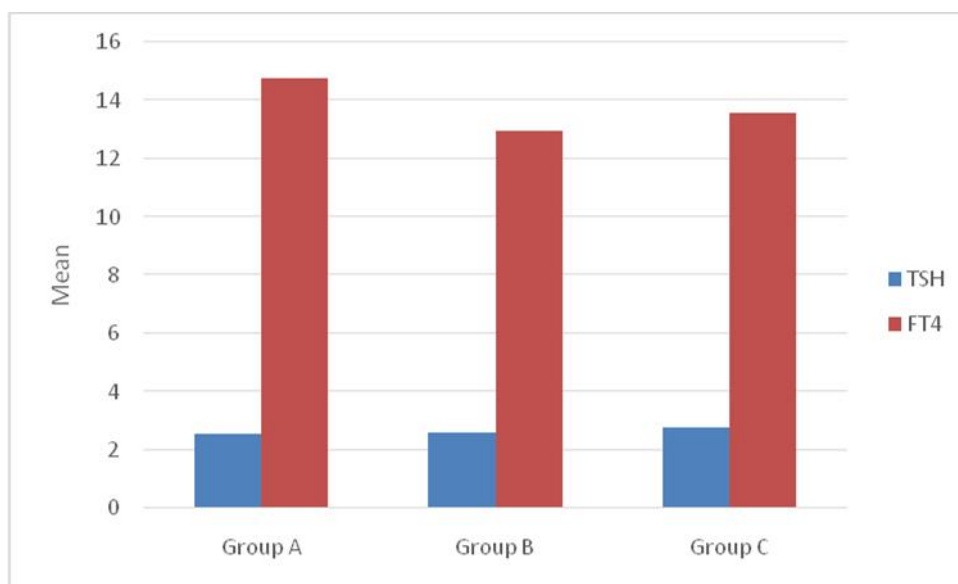
Significant differences in HB existed among the studied groups; Compared with group C, pregnant women in groups A and B had lower HB concentration (12.66±1.53 vs. 12.23±1.2 and 11.53±1.33 g/dl, P =0.01). HB concentration was lower in group B than group A (11.53±1.33 and 12.23±1.2 g/dl, respectively). Although serum ferritin level was higher in group C (non-pregnant) 31.27±33.02 in relation to its level in pregnant groups; 27.23±17.03 for group A and 24.64±19.71 for group B, no significant differences were found in serum ferritin level among the studied groups (P =0.33), table 2 & Figure 1.

Table (2) Comparison between the studied groups as regard TSH, FT4, HB, ferritin and vitamin B12:

	Group A	Group B	Group C	f-test	p
TSH Mean ±SD	2.57±1.44	2.59±1.11	2.76±2.88	X ² =2.79	0.25
FT4 Mean ±SD	14.77±2.13	12.94±1.89\$	13.56±2.89\$	X ² =14.74	<0.001**
B12 Mean ±SD	192.60±76.2	165.05±74.9	425.46±158.85\$#	X ² =139.0	<0.001**
Ferritin Mean ±SD	27.23±17.03	24.64±19.71	31.27±33.02	X ² =2.22	0.33
Hb Mean ±SD	12.23±1.2	11.53±1.33\$	12.66±1.53\$#	X ² =13.57	<0.01**

\$ Significance compared to group A, # Significance compared to group B and \$# Significance compared to group A&B.

Figure (1): Mean TSH and FT4 among the studied groups.



Following the guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy [11]. And according to the currently available report that included normal pregnant participants from the Gulf Region [12]; to assess the thyroid status among the studied groups, we found euthyroidism was much prevalent in non-pregnant (83.3%) than in pregnant women (53.8% and 50% for group A and group B respectively), while hypothyroidism (overt and subclinical) were more

prevalent in pregnant [(9.6% and 34.6% for group A) and (10.5% and 36.8% for group B)]. On contrary there were no hyperthyroidism in pregnant women and it was present in only 2.7% of the non-pregnant. On the other hand Hypothyroxinaemia had the same prevalence (2%) in group A and group B the pregnant women and it was absent in group C. However, there was significant statistical difference as regard thyroid status among the studied groups, $P = 0.001$, table 3.

Table (3) Thyroid status among the studied groups:

	Group A	Group B	Group C	FET	<i>p</i>
Euthyroid	56(53.8)	38(50.0)	62(83.8)	30.09	<0.001**
Hypothyroidism	10(9.6)	8(10.5)	2(2.7)		
Subclinical hypothyroidism	36(34.6)	28(36.8)	8(10.8)		
Hyperthyroidism	0(0.0)	0(0.0)	2(2.7)		
Hypothyroxinaemia	2(1.9)	2(1.9)	0(0.0)		

Correlation analysis of TSH with HB, ferritin and vitamin B12 levels in different studied groups revealed that ; in group A there were non-significant negative correlation between TSH level with HB and ferritin levels ($r = -0.17$ & -0.07 , $p = 0.09$ & 0.51 respectively), but there was highly significant positive correlation between TSH and vitamin B12 levels ($r = 0.25$, $p = 0.011$). In group B, TSH was negatively correlated with HB, ferritin and vitamin B12 levels, which was significant for HB and vitamin B12 and non-significant for ferritin ($r = -0.44$, -0.23 , -0.20 & $p = 0.001$, 0.044 , 0.08 , respectively). Also in group C, TSH was negatively correlated with HB, ferritin and

vitamin B12 levels, that was significant for HB and non-significant for ferritin and vitamin B12 ($r = -0.43$, -0.08 , -0.05 & $p = 0.001$, 0.49 , 0.66 , respectively). While on analysis of correlation of FT4 with HB, ferritin and vitamin B12 levels in different studied groups; we found that; in group A there were positive correlation between FT4 level with HB and ferritin levels, which was significant for HB and non significant for ferritin ($r = 0.27$ & 0.1 , $p = 0.006$ & 0.29 respectively), but there was non-significant negative correlation between FT4 and vitamin B12 levels ($r = -0.11$, $p = 0.26$). In group B, FT4 was positively correlated with HB, ferritin and vitamin B12 levels,

which was only significant with ferritin ($r = 0.15, 0.32, 0.04$ & $p = 0.2, 0.005, 0.71$, respectively). In group C there were positive correlation between FT4 level with HB and vitamin B12 levels, which was

significant for HB and non significant for vitamin B12 ($r = 0.28$ & $0.15, p = 0.017$ & 0.2 respectively), but there was non-significant negative correlation between FT4 and ferritin levels ($r = -0.16, p = 0.18$), table 4 & 5.

Table (4) correlation of HB, ferritin, vitamin B12 with TSH in different studied groups

TSH	Group A		Group B		Group C	
	r	p	r	p	r	p
HB	-0.17	0.09	-0.44	<0.001**	-0.43	<0.001**
ferritin	-0.07	0.51	-0.23	0.044*	-0.08	0.49
Vitamin B12	0.25	0.011*	-0.20	0.08	-0.05	0.66

Table (5) correlation of HB, ferritin, vitamin B12 with FT4 in different studied groups

FT4	Group A		Group B		Group C	
	r	p	r	p	r	p
HB	0.27	0.006**	0.15	0.20	0.28	0.017*
ferritin	0.10	0.29	0.32	0.005**	-0.16	0.18
Vitamin B12	-0.11	0.26	0.04	0.71	0.15	0.20

5. Discussion

Thyroid dysfunction is a worldwide phenomenon in women and the prevalence increases during pregnancy [13]. In most developing countries, nutritional deficiencies of nearly all essential nutrients, including iron, vitamin B12 and folic acid are common in pregnant women. Thyroid disorders and nutritional deficiencies especially of iron and vitamin B12 cause a number of maternofetal complications including the decreased ability of mother to cope up with bleeding that occur during delivery, low birth weight of fetus, growth retardation and increased perinatal mortality [14]. There are only a few reports of the prevalence of pregnancy-related thyroid disorders in the Saudi context. Therefore this study was conducted to assess thyroid status in pregnant Saudi women and its relation to ferritin and vitamin B12.

In this study we found non-significant lower TSH levels in first and second trimester of pregnancy when compared to controls, which was in agreement with *Zha et al* [5] who explored the range for thyroid hormones in normal pregnant Chinese and reported that TSH was significantly lower in pregnant than controls. And it was in contrast with the study of *Baghel et al* [15] who studied thyroid status in Indian pregnant women in the 1st trimester and found significantly elevated TSH level in pregnant than non-pregnant. And with the study of *Abdelhafiz et al* [16] who studied thyroid functions in Sudanese women in

mid and late pregnancy and also found significant elevation in TSH level in pregnant than non-pregnant. In our study mean TSH level was nearly similar in 1st and 2nd trimester 2.57 ± 1.44 and 2.59 ± 1.11 mIU, respectively which was slightly lower than mean TSH level in the study of *Chandrasekhara et al* [17] who studied thyroid disease in pregnant Indian women and found mean TSH level of 1st trimester 2.78 ± 2.33 mIU and in 2nd trimester was 2.82 ± 1.64 mIU.

In our study we found that serum FT4 levels in pregnant women decreased significantly from the first to the 2nd trimester and it is significantly higher in 1st trimester than in control group. Which was in accord with the study of *Zha et al* [5] who declared that serum FT4 levels in pregnant women decreased gradually from the first to the last three months. Our results were in contrast with the results of *Baghel et al* [15] who found FT4 level was significantly lower in 1st trimester pregnant women than controls.

The present study showed thyroid abnormalities in 16.2% among the non-pregnant participants and the most common thyroid disorders was subclinical hypothyroidism (10.8%) followed by overt hypothyroidism and hyperthyroidism having the same prevalence (2.7%). There were positive correlations of FT4 with (HB), while TSH correlated negatively with HB and ferritin. These findings were in accord with the study of *Refaat* [18] who study the prevalence and characteristics of anemia associated with thyroid

disorders in non-pregnant Saudi women; and found that thyroid disorders represent 19.6 % of the study population with subclinical hypothyroidism (59.3%) was the most prevalent followed by overt hypothyroidism (32.2%) and hyperthyroidism (8.4%), with also positive correlations of FT4 with (HB) and negative correlation of TSH with HB and ferritin. And also with study of *Hasanato et al [19]* who study the incidence of thyroid diseases in female Saudi adults in Riyadh and documented that hypothyroidism (15.5%) is much prevalent than hyperthyroidism(3%) in their studied group. And with study of *Al eidan et al. [20]* who found that subclinical hypothyroidism (10.7%) is more prevalent than subclinical hyperthyroidism (1.7%) in females participating in their study.

Our results documented thyroid abnormalities in (46.2%) and (50%) of pregnant females in 1st and 2nd trimester respectively and the most common thyroid disorder was subclinical hypothyroidism followed by overt hypothyroidism and hypothyroxinaemia. Furthermore, serum FT4 correlated positively with HB and ferritin, while TSH correlated negatively with HB and ferritin. Which were in agreement with *Refaat [13]* who study prevalence of pregnancy induced thyroid dysfunction and the characteristics of the associated anaemia in primigravida Saudi women during the first trimester in Mekkah. And with *Hussein [21]* who study prevalence of thyroid dysfunction among Saudi women in early pregnancy at King Abdulaziz University Hospital in Jeddah, who reported that hypothyroidism (40.3%) (Occult & overt) was the commonest thyroid dysfunction in 1st trimester in their studied group. In the study of *Shaheen and Hasan [14]* who explore the prevalence of anemia and its effects on thyroid function in pregnant Indian women they also found that thyroid abnormalities represent 50% of their studied group(anaemic and non- anaemic) with subclinical hypothyroidism was the most prevalent , when the prevalence of thyroid disorder was compared among the pregnant women anaemic and non-anaemic , the prevalence rate was much higher in pregnant anaemic women suggesting strong association between hypothyroidism and iron deficiency. Our results were also coincident with the study of *Ahmed et al. [22]* who compare the universal and targeted screening for thyroid dysfunction in pregnant Egyptian women, and found that hypothyroidism is the most common dysfunction (55.9%) with subclinical hypothyroidism represent 42.8% and overt hypothyroidism represent 13% in their studied group (low and high risk), with the similar prevalence in 1st, 2nd and 3rd trimesters.

Lower TSH level in pregnant than non-pregnant together with high FT4 level in the 1st trimester than in 2nd trimester and controls, and the increased prevalence of hypothyroidism (being the commonest disorder) in our study were due to the physiological and hormonal changes occurring in normal pregnancy that alter thyroid function. The increased glomerular filtration rate which occurs in pregnancy can lead to increased losses of urinary iodine, resulting in iodine deficiency and eventually maternal goiter. Thyroid function tests change during pregnancy due to the influence of two main hormones: human chorionic gonadotropin (hCG) and estrogen. The high circulating hCG levels in the first trimester may result in a slightly low TSH. When this occurs, the TSH will be slightly decreased in the first trimester. Estrogen increases the amount of thyroid hormone binding proteins in the serum which increases the total thyroid hormone levels in the blood since >99% of the thyroid hormones in the blood are bound to these proteins. In sum, pregnancy-induced stress on the thyroid can lead to hypothyroidism.

Moreover, we found significantly lower levels of hemoglobin and vitamin B12 levels in first and second trimester of pregnancy when compared to controls. Also ferritin was non- significantly lower in pregnant than non pregnant women. Which was in accord with the study of *Baghel et al [15]* who reported that vitamin B12 and iron were significantly lower in the 1st trimester of pregnancy than in non-pregnant. In our study we had noticed that vitamin B12 was positively correlated with TSH in the 1st trimester and negatively correlated with TSH in the 2nd trimester, which was different from the study of *Bashetti et al [10]* who study the association of vitamin B12 and folic acid with thyroid hormones in pregnant Indian women with hypothyroidism and found TSH was correlated negatively with vitamin B12 in 1st & 2nd trimester, but these might be due to that all participants in their study were hypothyroid. However, vitamin B12 is very essential in all stages of life and its demand increases during pregnancy, fetal development and infant growth states the dependency of the fetus on maternal vitamin B12 for proper development and growth.

The first trimester pregnancies without any supplementation more likely reflect the preconceptional status. Our results emphasize that, iron and B12 deficiency were common problem in this geographical region. These pregnant women will probably develop iron and B12 deficiency anemia with

the progression of pregnancy. Screening in the preconceptional period or early pregnancy seems valuable for the detection and treatment .

6. Conclusion

In conclusion, we study maternal thyroid status in 1st & 2nd trimester in pregnant women in Northern Area, KSA and its relation to ferritin and vitamin B12. We observed a lower TSH level in pregnant than non-pregnant. We found that subclinical hypothyroidism was the most common thyroid disorder followed by overt hypothyroidism and hypothyroxinaemia. In addition, we show a lower levels of hemoglobin, ferritin and vitamin B12 levels in first and second trimester of pregnancy when compared to controls. Serum FT4 correlated positively with HB and ferritin, while TSH correlated negatively with HB and ferritin. So, these results highlight the urgent need for thyroid status to be detected early and treatment started promptly. Therefore, screening pregnant women for maternal thyroid dysfunction as early as possible should be considered, particularly in a country like Saudi Arabia.

More researches are needed to be conducted so that a clear link associated with thyroid function and iron status can be ruled out in order to prevent the adverse effects of the disorder to both mother and foetus so as to achieve normal pregnancy outcome. Also, we recommend further detailed, multi centre studies to understand the association between vitamin B12, and thyroid profile. So, early diagnosis of these deficiencies will be useful to start giving supplements to avoid unwanted effects in pregnancy.

7. Study Limitation

However, our study has a few limitations. First, we did not investigate autoimmune thyroid disease as a cause of thyroid disorder. Second, the study should be extended to the population in iodine deficient or excessive area. Further research is needed to determine the role of maternal thyroid dysfunction with iron and vitamin B12 deficiency in fetal development. Lastly, our study was conducted in one center while it is better to be multicenter study.

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