Received: 27 August 2022 | Revised: 13 September 2022 | Accepted: 13 September 2022
DOI: 10.22034/ecc.2023.359380.1527
Eurasian Chemical Communications
SAMI

FULL PAPER

Correlations of serum transthyretin and thyroid hormones with other biochemical parameters in Iraqi pregnant women who undergo spontaneous miscarriage

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Spontaneous abortion or miscarriage refers to the loss of a clinically confirmed pregnancy prior 20 weeks of gestation. Reproductive function before, during, or after pregnancy can be greatly affected by changes in thyroid function. Thyroid disease is the most common endocrine condition affecting the women of reproductive age. This study was conducted on Iraqi women with age (20-35) years old who suffered from the spontaneous miscarriage. The study included two groups of Iraqi patients, where the first group (P1) refers to patients (31) with incomplete abortion and the second group (P2) refers to patients (29) with complete abortion. For comparison, twenty-eight Iraqi women control subjects group(C). The results showed significant increase of tetraiodothyronine (thyroxine T4) levels at (P<0.05) between P1 group compared with C group and a highly significant difference at (P<0.01) of transthyretin (TTR) levels between P1 group in comparison with C groups. Furthermore, a significant increase of thyroid-stimulating hormone (TSH) levels of P1 group, as compared with C groups (P=0.013). Moreover, the progesterone and estradiol levels showed a highly significant difference at (P< 0.01) between P1 and P2 groups, and against C group. The study concluded that T4, TSH, and TTR levels were significantly higher in pregnant women with spontaneous abortions compared with normal pregnant women. Furthermore, serum estradiol and progesterone levels were substantially lower in women with spontaneous miscarriage than in healthy pregnant women. Thus, biochemical screening for thyroid and reproductive hormones is essential in early pregnancy to reduce the risk of miscarriage. The current study revealed that the maternal thyroid dysfunction negatively affects pregnancy. It also demonstrates that maternal serum hormones differed significantly between normal pregnant individuals and women who miscarried, and the required level was diagnosed to maintain pregnancy and reduce the miscarriage risk.

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KEYWORDS

Spontaneous miscarriage; abortion, gestational; pregnancy; transthyretin; thyroid hormones.

Introduction

A miscarriage is a pregnancy loss that occurs spontaneously the 20th week of pregnancy without resorting to the use of surgical or therapeutic methods to get rid of pregnancy [1]. It happens in 10 to 15 percent clinically diagnosed pregnancies, with eighty percent of miscarriages occurring prior to the 12th week

of pregnancy and miscarriage rates drastically drop following the first trimester [2]. Miscarriage can occur for different reasons including autoimmune disorders, chromosomal abnormalities, uterine abnormalities, infections, and endocrinological abnormalities [3]. Thyroid hormones have been further discovered to be important for healthy brain maturation, growth, and function. The secretion of critical cytokines and angiogenic growth factors by human decidual cells is regulated by the optimal maternal thyroid hormone concentrations, preventing fetal immune rejection, and promoting normal placental development. It was therefore hypothesized that thyroid hormones may be crucial for preserving a balanced inflammatory response in the early stages of pregnancy [4]. Thyroid diseases can affect the health of the mother and fetus during pregnancy, especially hypothyroidism, which is more prevalent than hyperthyroidism (2.5% vs. 0.2%) during pregnancy and has a statistically significant link to recurrent first-trimester pregnancy losses [5].

According to the previous studies, the main hormones secreted by the thyroid gland are predominantly tetraiodothyronine (thyroxine: T4) and triiodothyronine (T3), where 85% of T3 is produced from T4 in the blood circulation and other organs such as the liver, heart, kidney, and muscles. After hormone secretion, T4 stays in the blood longer than T3 (~ 1 week compared with ~18 hr for T3). These hormones are controlled by another hormone secreted by the anterior pituitary gland, which is called thyroid-stimulating hormone (TSH) [6]. The pituitary gland secretes TSH, which is then released into the bloodstream. By interacting with receptors on thyroid gland cells, it regulates production thyroid gland's hormones, thyroxine (T4) and triiodothyronine (T3), these hormones are necessary for the body's metabolism, cardiac, and digestive processes, muscle control, brain development, and bone maintenance [7].

Transthyretin (TTR or TBPA) is a transport visceral protein, formerly known as thyroxine-binding prealbumin. It is a transport protein in the serum and cerebrospinal fluid carrying the thyroid hormone thyroxine (T4) and retinol-binding protein-bound to retinol. This is how transthyretin gained its name transports thyroxine and retinol [8]. This study aims to study the effect of different levels of thyroid hormones during pregnancy and their impact on the mother and fetus, and to help reduce the miscarriage risk as a result of these changes.

Materials and methods

Study groups

This study was conducted on women who suffered from spontaneous miscarriage, the study included two groups of Iraqi patients, the first group (P1) refers to patients (31) with incomplete abortion and the second group (P2) refers to patients (29) with complete abortion. For comparisons, twenty-eight Iraqi women control subjects (group C), the selection of the controls was based on choosing the apparently healthy (pregnant women with normal pregnancy within the first 24 weeks of GA). Patient samples were collected from Al-Elwea Hospital for Obstetrics and Gynecology, Baghdad, Iraq.

Exclusion criteria

The pregnant women with threatened abortion, pregnant women with history of medical diseases including (D.M, hypertension, thyroid disorders, other endocrinal disorder, and antiphosolipid antibodies) and pregnant women with GA > 24 weeks, were excluded from the current study.

Blood sample collection

(8 mL) of the blood was obtained by venipuncture from each control and patient

subject collected from Al-Elwea Hospital for Obstetrics and Gynecology, divided into three parts. The first one (3 mL) was dispensed in a gel tube and left to clot at room temperature. The gel tube was centrifuged at 3000 ×g for 10 minutes to collect serum used for measurements of the estradiol (E2), progesterone, Triiodothyronine (T3), T4, TSH, TTR, and blood glucose. While the second part (2 mL) was transferred into citrate containing tube to be used for hematological measurement of erythrocyte sedimentation rate (ESR) and the third part (3 mL) was transferred into tube containing ethylene diamine tetra acetic acid (EDTA) tube used for the HBA1C and CBC estimation.

Anthropometric measurements

The body mass index (BMI) of the studied subjects was calculated by the applying following equation:

$$\text{BMI}=\text{Weight (kilograms)}/\text{Height (meters)}^2$$

The normal range of BMI was reported between 18 to 24.9 Kg/m^2, a BMI lower than 18.5 Kg/m^2 classified as underweight, BMI between (25 to 29.9) Kg/m^2 classified as overweight and 30 Kg/m^2 or more were classified as obese [9].

Laboratory assay

Serum glucose level was measured by using Cobas C 111 Analyzer-Roche diagnostics device. (TSH) Serum thyroid-stimulating hormones, (TT3) total tri-iodothyronine, (TT4) total thyroxine, (TTR) transthyretin, (E2) estradiol, (Prog) progesterone level were analyzed by using enzyme-linked immunosorbent assay (ELISA) technique by using the commercially available ELISA kit (elabscience, the USA) and (Abnova, Germany). All ELISA procedures were carried out in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed by using IBM SPSS statistics version 26. The variables were reported as means ± standard deviation. The groups were compared by using one-way ANOVA. The parameters of both the controls and the cases were compared by using Karl Pearson's correlation. Likewise, the control group was used to compare the mean values of several groups to find the statistical significant changes. A statistical comparison of the total data was done (i.e. 28 controls, and 31 cases incomplete and 29 cases complete). Statistically, the significant difference was considered for all tests at (P< 0.05) and highly significant difference at (P< 0.01).

Results and discussion

All anthropometric data obtained from patients and control groups are summarized in Table (1). The mean ± SD of age of the patients was (29.64±5.22) and (27.00±7.28) years old for the P1 and P2 groups, respectively, which are non-significant compared with the mean age of the control group (26.75±4.85). The mean±SD of ESR of the patients was (23.428±6.257 mm/hr) for the P1 group showed a highly significant difference at (P<0.01), compared with the control group (14.608±4.309 mm/hr), as indicated in Table (1). The RBS levels of P1 group was (98.193±12.012 mg/dl) and (96.517±10.479mg/dl) for P2 group, which are non-significant, as compared with the values of the control group (91.907±16.143 mg/dl). The mean±SD value of HbA1C of the P1 group was (5.156±0.440) and (5.205±0.434) for the P2 group, respectively, which are non-significant compared with the control group (5.027±0.447). The results of complete blood count (CBC) HB and HCT mean levels showed a highly significant differences (P<0.01) between P1, P2 and C groups. The PLT mean levels in P1 group (219.41±28.77 u/l) indicated a highly significant increase, as compared with C group (281.64±41.25 u/l) with significant differences at (P<0.01).

However, P2 group showed a significant difference (P<0.05) as compared with C group. In the contrast, the WBC mean levels did not demonstrate any significant differences (P=0.067) between P1 and P2 with C groups. The purpose of designing this study was to examine the levels of different hormones and to assess the relationship between them, if any, between the hormones of different cases and controls. The results of the different groups are reported in Table 1. It was observed that significant increase in T4 mean levels of group P1 in comparison to C group (109.87±15.53 nmol/l) and (96.87±16.22 nmol/l), respectively. A highly significant difference at (P<0.01) in TTR mean levels between P1 group as compared with C groups, a highly significant difference between P1 and P2 was observed in the TTR comparison. Moreover, the current study revealed a significant increase in TSH means levels of P1 group in comparison to C groups (P=0.013)), and also a significant difference between P1 and P2 was observed in the TSH comparison. The PROG and estradiol levels showed a highly significant difference at (P< 0.01) between P1 and P2 groups, against C group. AS the mean ± SD value of PROG and estradiol of the patients was (15.615±5.099 ng/mL, 339±94 pg/mL, respectively) for the P1 group (11.737±3.815 ng/mL, 250±45 pg/mL, respectively) for P2 group, which are significant compared with the values of the control group (28.153±14.220 ng/mL, 1036±115 pg/mL, respectively), as presented in Table 1.

TABLE 1 Mean (±SD) level of different parameters for groups C, P1, and P2

Parameters	C (N=28)	P1 (N=31)	P2 (N=29)	P-value
BMI	25.52±3.05	27.39±2.45	26.62±2.39	0. 281
Age (year)	26.75±4.85	29.65±5.23	27.01±7.28	0.113
ESR (mm/hr)	14.61±4.31	23.43±6.26^{**a}	19.15±8.06	<0.01
RBS (mg/dl)	91.91±16.14	98.19±12.01	96.52±10.48	0.170
HbA_1C (%)	5.03±0.45	5.16±0.44	5.21±0.43	0.294
HB (%)	12.55±0.64	11.04±0.59^{**a}	11.05±0.65^{**b}	<0.01
HCT (g/dl)	37.69±1.90	32.41±1.68^{**a}	32.75±1.99^{**b}	<0.01
WBC (u/l)	7.36±1.46	7.86±1.54	6.86±1.88	0.067
PLT (u/l)	281.64±41.25	219.41±28.77^{**a}	243.79±58.65^{*b}	<0.01
T3 (nmol/l)	2.02±0.59	1.91±0.54	1.87±0.45	0.557
T4 (nmol/l)	96.87±16.22	109.87±15.53^{*a}	104.23±20.51	<0.05
TSH (µui/mL)	1.77±0.49	2.27±0.82^{*a,c}	1.82±0.76^{*c}	<0.05
TTR (ng/mL)	559.91±142.63	807.44±149.15^{**a,c}	593.37±150.84^{**c}	<0.01
E2 (pg/mL)	1036±115	339±94^{**a}	250±45^{**b,c}	<0.01
PROG (ng/mL)	28.15±14.22	15.62±5.09^{**a}	11.74±3.82^{**b}	<0.01

Results were expressed as mean±SD, ANOVA test was used for comparison between the three groups. *P< 0.05 is significant, **P<0.01 is highly significant, and no asterisk: P> 0.05.
(a): It refers to the significant difference between C and P1, (b): It refers to the significant difference between C and P2, and (c): It refers to the significant difference between P1and P2.
T3=Triiodothyronine, T4=Thyroxine, TSH=Thyroid stimulating hormone, RBS=random blood sugar, BMI=The body mass index, PROG= progesterone, E2= estradiol, and TTR= Transthyretin.

Table 2 indicates Pearson correlation coefficient of serum TSH, T3, and T4 in incomplete abortion P1 group. Based on the results, the serum T3 level had a positive correlation with serum E2 (P=0.043). The serum TSH had positive correlation with HbA1C (P=0.010).

Table 3 demonstrates Pearson correlation coefficient of serum TSH, T3, and T4 in complete abortion P2 group, the serum T3 level had a positive correlation with HbA1C (P=0.039) and a high significant positive correlation with serum E2(P=0.001). The T4 had no correlation with any parameters, while the serum TSH level had positive correlation

with HbA1C (*P*=0.01) and a negative significant correlation with serum progesterone (*p*=0.050).

Table 4 illustrates Pearson correlation coefficient of serum TTR. The serum TTR had no correlation with any parameters in both groups of patients.

TABLE 2 Pearson correlation analysis of thyroid hormones with other parameters group P1

Parameters	P1 Group (n=31)								
	T3			T4			TSH		
	R	*P*	Sig.	R	*P*	Sig.	R	*P*	Sig.
Merntalage	0.408	0.056	NS	0.029	0.877	NS	-0.11	0.557	NS
ESR	0.197	0.287	NS	-0.031	0.866	NS	-0.21	0.909	NS
HbA1C	-0.016	0.931	NS	0.076	0.686	NS	0.454	0.010*	S
RBS	0.207	0.265	NS	0.054	0.773	NS	-0.121	0.518	NS
HB	0.169	0.363	NS	0.013	0.946	NS	0.211	0.254	NS
HCT	0.221	0.232	NS	0.032	0.863	NS	0.097	0.602	NS
WBC	-0.161	0.386	NS	0.112	0.550	NS	0.029	0.877	NS
PLT	-0.210	0.257	NS	-0.168	0.367	NS	-0.234	0.206	NS
E2	0.366	0.043	S	0.277	0.132	NS	-0.151	0.416	NS
PROG	-0.011	0.955	NS	0.025	0.894	NS	0.044	0.814	NS

HS: P< 0.01, S: P< 0.05, and NS: P> 0.05

TABLE 3 Pearson correlation analysis of thyroid hormones with other parameters group P2

Parameters	Group P2 (n=29)								
	T3			T4			TSH		
	r	*p*	Sig.	r	*p*	Sig.	r	*p*	Sig .
Merntalage	0.187	0.333	NS	-0.097	0.617	NS	0.032	0.867	NS
ESR	-0.126	0.516	NS	-0.107	0.581	NS	0.033	0.867	NS
HbA1C	0.386	0.039*	S	0.218	0.255	NS	0.307	0.01*	S
RBS	-0.150	0.436	NS	0.076	0.697	NS	-0.229	0.231	NS
HB	-0.047	0.809	NS	-0.094	0.626	NS	0.191	0.322	NS
HCT	-0.043	0.826	NS	-0.134	0.488	NS	0.146	0.450	NS
WBC	-0.173	0.368	NS	0.068	0.725	NS	-0.047	0.810	NS
PLT	0.114	0.557	NS	-0.133	0.491	NS	-0.139	0.471	NS
E2	0.622	0.001	HS	0.028	0.884	NS	-0.246	0.199	NS
PROG	0.264	0.167	NS	-0.239	0.211	NS	-0.333	0.050*	S

HS: P< 0.01, S: P< 0.05, and NS: P> 0.05

TABLE 4 Pearson correlation analysis of TTR with other parameters in P1 and P2

Parameters	Group P1 (N=31)			Group P2 (N=29)		
	R	P	Sig.	R	P	Sig.
ESR	-0.027	0.887	NS	-0.363	0.053	NS
RBC	0.167	0.368	NS	-0.054	0.780	NS
HbA1C	0.109	0.559	NS	-0.153	0.429	NS
PROG	-0.189	0.309	NS	-0.327	0.084	NS
HCT	0.336	0.065	NS	-0.102	0.598	NS
HB	0.233	0.207	NS	-0.103	0.595	NS
WBC	-0.041	0.826	NS	-0.160	0.406	NS
PLT	0.005	0.978	NS	0.073	0.706	NS
E2	0.256	0.164	NS	0.133	0.492	NS
Merntalage	0.029	0.877	NS	-0.097	0.617	NS

HS: P< 0.01, S: P< 0.05, and NS: P> 0.05

Thyroid disease is the most common endocrine condition affecting women of reproductive age. The developing fetus depends on thyroid hormones for survival, yet the thyroid gland of the fetus does not produce these hormones not be perfectly functional until 12 weeks into the pregnancy. Therefore, there is a higher risk of miscarriage if the mother does not have enough thyroid hormones [10]. Thyroid autoimmunity (TAI) is defined as the autoantibodies existence against thyroid peroxidase associated with spontaneous miscarriage [11]. Thyroid hormones may have a role in controlling apoptosis at the trophoblast level. They have been demonstrated to inhibit apoptosis by decrease regulating the expression of fas and fas ligand in the early placental extravillous trophoblast [12]. Furthermore, *in vitro* evidence demonstrates that thyroid hormone up-regulates integrin expression, which in turn affects the invasiveness and differentiation of cultured extra villous trophoblast cells and metalloproteases supports the importance of enough thyroid hormone concentration for a normal placentation process [13].The results of our study revealed that the serum level of T3 is lower in spontaneous miscarriage patients P1and P2 groups, as compared with control. On the other hand, serum T4, TSH and TTR values were elevated in spontaneous miscarriage patients compared with control. Our results are consistent with prior research suggesting that the thyroid markers levels were altered in miscarried pregnant women compared with those who had normal pregnancies [13,14] compatible with the concept of thyroid autoantibodies as a sign for an unknown autoimmune. Accordingly, the still unidentified autoimmune is blocking TSH receptor autoantibodies, which may putatively suppress the action of human chorionic gonadotropin (βHCG) on the corpus luteum through a cross-reactivity mechanism [12,15]. This inhibition may result in a reduction in progesterone, placental lactogen and estrogen production, both of which are necessary for the support and maintenance of pregnancy throughout the first trimester [16]. As a result, an insufficient supply of thyroid hormone at the trophoblast level might result in abnormal trophoblast endocrine function. Hence, it is possible that elevated TSH levels are a factor in the higher risk of miscarriage in women who have thyroid antibodies [14]. Thyroid hormones have an important role in all stages of pregnancy, as has been widely reported. These hormones can alter trophoblast endocrine activity in the early stages of pregnancy by directly stimulating the synthesis and secretion of progesterone and estradiol [16]. Furthermore, the recent studies revealed that serum progesterone [17,18] and estradiol [20] can identify women with miscarriage. The immune cells and estradiol of pregnant females can cooperate to maintain a healthy pregnancy [19]. Estradiol levels and low growth rates are likely to indicate a bad pregnancy outcome [20] significantly lower estradiol level in women with spontaneous miscarriage than in control [19]. Our results are consistent with other studies that suggested estradiol levels were lower in pregnant women with miscarriage than in women who with healthy pregnancies [21]. The results of our study revealed that serum progesterone is significantly lower in spontaneous miscarriage patients than in control. Our results are in agreement with other studies that suggested progesterone levels were lower in women with abortions than in those with normal pregnancies [17,18,22]. Progesterone can provide endocrine support. Another study reported the high progesterone levels to have a calming impact, decrease the strength and frequency of uterine contractions, and thereby extend the endometrial secretion period and embryonic development in planting window synchronization [23]. It was

illustrated that progesterone can enhance immunological status by stimulating the release of progesterone-induced blocking factor (PIBF) by CD56+ lymphocytes at the maternal-fetal interface preventing immune rejection [24]. Progesterone levels may confirm a diagnosis of embryonic development. According to another study, the serum progesterone levels less than 6.2 ng/mL suggest decreased embryonic vitality. The specificity and sensitivity for detecting an early loss of pregnancy were 20% and 100%, respectively [24].

Conclusion

The current study revealed that the maternal thyroid dysfunction negatively affects pregnancy. It also reveals that maternal serum hormones differed significantly between normal pregnant individuals and females who miscarried, and the required level was diagnosed to maintain pregnancy and reduce the miscarriage risk.

Acknowledgements

Support of this investigation by Baghdad University, collage of Science and Al-Elwea Hospital for Obstetrics and Gynecology Foundation is gratefully acknowledged.

Conflict of Interest

The authors declare that there is no conflict of interest.

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How to cite this article: Nabaa L. Majeed, Namir I.A. Haddad*, Ekhlas A. Hussein. Correlations of serum transthyretin and thyroid hormones with other biochemical parameters in Iraqi pregnant women who undergo spontaneous miscarriage. *Eurasian Chemical Communications*, 2023, 5(2), 136-143. **Link:** http://www.echemcom.com/article_158376.html