

Association of Serum Proteins Electrophoretic Pattern and Serum Hormones in Women with Spontaneous Pregnancy Loss

Abstract

Background: Pregnancy is the state of carrying a developing embryo or fetus within a female body. Once pregnancy is established, a range of endocrinological events appear in its maintenance, finally helping in the successful pregnancy. The complications which are usually observed in pregnancy are gestational diabetes, preeclampsia, preterm labor, and spontaneous pregnancy loss or miscarriage, while 10%–15% of clinically recognized pregnancies terminate into spontaneous miscarriage. Thus, many attempts have been made by different researchers for the diagnosis of high-risk pregnancy on altered protein pattern using placental villous tissue or follicular fluid, but these are difficult to obtain and results of different studies are not constant. **Aim:** This study was designed to identify the association (if any) among serum protein(s) electrophoretic pattern and different serum hormones in normal pregnant women (controls) and gestational age-matched women with spontaneous pregnancy loss (cases). **Materials and Methods:** This study was carried out for 1½ year from October 2018 to March 2020 and included 120 participants (60 normal pregnant women and 60 women with spontaneous pregnancy loss) between 20 and 45 years of age with no mean age difference. The electrophoresis of serum was carried out using slab gel electrophoretic unit and serum thyroid-stimulating hormone (TSH), total tri-iodothyronine (TT₃), total thyroxine (TT₄), prolactin, and beta human chorionic gonadotropin (β-hCG) levels were analyzed using TSOSH AIA analyzer at Adesh University, Bathinda. **Results:** Significant variations in the expression of proteins with molecular weight around ~150 kDa, ~50 kDa, and ~25 kDa were observed in normal pregnant women and women with spontaneous pregnancy loss. However, the protein band of ~50 kDa was found to be highly expressed in the serum of 1st and 2nd trimester women experiencing spontaneous pregnancy loss. Therefore, selected protein band of ~50 kDa was further processed by ECI-mass spectrophotometry QUAD time of flight and 365 different proteins were found, out of these; 34 proteins were found to be unidentified protein products (*Verified using NCBI data base*). Further, TT₃, total proteins, β-hCG, and prolactin level were found to be low, whereas, TSH was found to be high in women experiencing spontaneous pregnancy loss. However, difference in the level of β-hCG in the 1st trimester and TT₄ among normal pregnant women and women with spontaneous pregnancy loss was observed to be statistically insignificant. **Conclusion:** This study indicated that the evaluation of serum protein variations along with hormonal profile may provide valuable information about high-risk pregnancy. Moreover, the differential expression of proteins in women with spontaneous pregnancy loss can be further explored to develop potential biomarker for the early identification of high-risk pregnancy and appropriate preventive measure.

Keywords: Hormones, miscarriage, pregnancy, serum electrophoretic protein pattern

Introduction

Pregnancy is a complex biological process where after conception; a fetus is formed as an embryo from a single cell.^[1] Once pregnancy is established, the fetus, placenta, and mother initiate and maintain communication by means of endocrine system. A range of endocrinological events appears in its maintenance; finally helping in the successful pregnancy.^[2] However, some women experience health complications

during pregnancy, which can involve the mother's health, the fetus's health, or both. Gestational diabetes, preterm labor, preeclampsia, pregnancy loss, or miscarriage are the common complications observed in pregnancy.^[3] Miscarriage is a common complication encountered during early pregnancy with the reports that each year an estimated 210 million women throughout the world become pregnant and about one in five of them resort to miscarriage.^[4] As per the UNICEF, 800 women, globally die every day due to

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abnormalities related to childbirth and pregnancy. Out of those 800 women, 160 women are from India. However, different studies have demonstrated that actual rate of miscarriage might be higher as many women may have very early miscarriages without even realizing that they are pregnant.^[5] Previous studies have suggested that 80% of miscarriages occur before 12 weeks of gestation.^[6] During gestation, alterations in the hormones are well documented and thyroid diseases have been diagnosed with the second most common endocrinological disorder in women of childbearing age.^[7] Since, proteins or peptides are known as the building blocks of most hormones and many important biological components, thus abnormal protein or peptides homeostasis and expression may result in alterations in maternal hormonal profile and may be the reason for spontaneous pregnancy loss. Therefore, this study was oriented toward the evaluation of different serum hormones and identification of altered serum electrophoretic pattern between normal pregnant women and women experiencing spontaneous pregnancy loss.

Materials and Methods

This study was carried out for 1½ year from October 2018 to March 2020 after approvals from the institutional research committee and ethics committee of Biomedical and Health Research (AU/EC/FM/158/2018).

In this study, 120 participants, i.e., 60 confirmed cases of spontaneous pregnancy loss (cases) and 60 gestational age-matched normal pregnant women (controls) before 20 weeks of gestation were included. In 60 confirmed cases of spontaneous pregnancy loss, 35 participants experienced pregnancy loss in the 1st trimester, while 25 were of the 2nd trimester. The age of the participants was between 20 and 45 years with no mean age difference in the 1st and 2nd trimesters between normal pregnant women and women experiencing spontaneous pregnancy loss. Participants attending the outpatient department and inpatient department of Obstetrics and Gynecology, Adesh hospital, Bathinda were included based on the inclusion criteria (cases: clinically confirmed cases of spontaneous pregnancy loss/miscarriage, controls: gestational age-matched pregnant individual, no previously recognized miscarriage; no clinical history of endocrine abnormality), and exclusion criteria (patients with a history of thyroid dysfunction, diabetes mellitus, polycystic ovary syndrome, heart disease, uterine fibroid, infectious disease, or participant rejecting the consent). The sample size was determined by using the mean and standard deviation from the results of previous study of our laboratory.^[8] The sample size was calculated using the sample size formula, comparing two means and verified by using the online sample size calculator (openepi.com version 3.01) at two-sided confidential interval at 95%.

Prior to enrollment, all the necessary details about this study were provided to participants in the language which they

understood best and informed written consent was obtained. All the detailed history, including age, age at marriage, last menstrual cycle, age at first pregnancy, and obstetrics history with full clinical examination, was recorded. 03 ml of blood was drawn within 3 days of miscarriage from the women with spontaneous pregnancy loss and from gestational age-matched normal pregnant women. The serum samples were separated and preserved at -20°C. The total protein content of serum was determined by Biuret method using spectrophotometer at 550 nm. TSOSH automated immunoassay analyzer was used to analyze serum TSH, total tri-iodothyronine (TT₃), total thyroxine (TT₄), prolactin and beta human chorionic gonadotropin (β-hCG) level, and serum was subjected to Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) by vertical slab gel SDS-PAGE using 5% stacking over 12% resolving gel in Bio-Rad unit at Centre for Interdisciplinary Biomedical Research, Adesh University. After 1-D electrophoresis and staining the gel with Coomassie brilliant blue R-250, protein band was cut and sent for electrospray ionization mass spectrophotometry (ESI-MS) quad time of flight (TOF) at Advanced Technology Platform Centre of Regional Center for Biotechnology (RCB), Faridabad, where trypsin digestion of protein was carried out and peptides were analyzed by ESI-MS QUAD TOF. The mass profiling of the peptides was received in mascot format and identified protein sequences were compared using the blast tool from NCBI database.

Statistical analysis

Statistical analysis was performed using the Microsoft Excel. Necessary statistical tools were applied for the correlation and comparison of different results. For all statistical tests, 95% confidence interval was taken into consideration, i.e., $P \leq 0.05$ was considered statistically significant.

Results

The serum total proteins of normal pregnant women and women with spontaneous pregnancy loss at the 1st and 2nd trimesters resolved on 12% denaturing gel showed the presence of protein bands ranging from 250 kDa to 10 kDa. For the better resolution, the concentration of serum protein of different samples was estimated using spectrophotometer and different volume of sample having the same concentration of proteins was loaded into each well of gel and resolved under denaturing conditions. Bio-Rad protein marker was used to compare and to estimate the tentative molecular weight of the protein bands resolved on SDS-PAGE.

Aligned with protein marker, many protein bands in sera of normal pregnant women and women experiencing spontaneous pregnancy loss in the 1st trimester were found to be differently expressed. A protein band of ~150 kDa was found to be highly expressed in normal pregnant women and was visible as strong dark band, while in

women with spontaneous pregnancy loss, band was lightly expressed. Out of 35 normal pregnant women and women experiencing spontaneous pregnancy loss of the 1st trimester pregnancy, this protein band of ~150 kDa was highly expressed in 26 (74.28%) normal pregnant women, while in only 09 (25.71%) women experiencing spontaneous pregnancy loss, it showed a weak intensity of expression.

Similarly, in the region around 50–55 kDa, a protein band of ~50 kDa appeared to be differently expressed between normal pregnant women and women experiencing spontaneous pregnancy loss. The protein band seemed to be highly expressed in women experiencing pregnancy loss, appearing as strong dark band, while in women with normal pregnancy, it was lightly expressed or completely missing. Out of 35 normal pregnant women and women experiencing spontaneous pregnancy loss, this protein band of ~50 kDa was highly expressed in 31 (88.57%) women experiencing spontaneous pregnancy loss, while in only 04 (11.42%) women experiencing spontaneous pregnancy loss and 3 normal pregnant women (8.57%), weak intensity of protein band expression was observed.

In low-molecular-weight proteins, a protein band around ~25 kDa was observed to be highly expressed in women experiencing spontaneous pregnancy loss and visualized as strong dark band, whereas in normal pregnant women, it was lightly expressed or was missing. Out of 35 normal pregnant women and women experiencing spontaneous pregnancy loss, this protein band of ~25 kDa was highly expressed in 28 (80%) women experiencing spontaneous pregnancy loss, while in 07 (20%) women experiencing spontaneous pregnancy loss, it was lightly expressed.

Further, protein expression in women with normal pregnancy and women experiencing spontaneous pregnancy loss in the 2nd trimester was compared and result showed differential expression of proteins in the serum of 2nd trimester too. The protein band of approximately ~50 kDa was also found to be differently expressed between normal pregnant women and women experiencing spontaneous pregnancy loss in the 2nd trimester. This band was highly expressed in women experiencing spontaneous pregnancy loss, visualized as strong dark band, while in normal pregnant women; it seemed to be weakly expressed or not expressed. Out of 25 normal pregnant women and women experiencing spontaneous pregnancy loss, this protein band of ~50 kDa was highly expressed in 21 (84%) women experiencing spontaneous pregnancy loss, while, in 04 (16%) women experiencing spontaneous pregnancy loss and 2 normal pregnant women (8%), it was lightly expressed. Further, in low-molecular-weight proteins, ~25 kDa protein band was also highly expressed in women experiencing spontaneous pregnancy loss and visualized as strong dark band, while in normal pregnant women, it was lightly expressed.

However, variations in the other protein bands were not observed in the participants of the 2nd trimester.

For further confirmation of differential expression of the protein bands, SDS PAGE gel was stained with Coomassie Brilliant Blue R-250 and the variations in the protein bands of approximately 50 kDa and 25 kDa were again observed [Figure 1].

Therefore, the findings suggested that there are prominent variations in the expression of serum proteins in women with spontaneous pregnancy loss as compared to normal pregnant women. However, the protein band of ~50 kDa was found to be highly expressed in a greater number of 1st (31, i.e., 88.57%) and 2nd trimesters (21, i.e., 84%) women experiencing spontaneous pregnancy loss, therefore, this protein band of ~50 kDa was selected for the identification of differential protein expression in women experiencing spontaneous pregnancy loss and was sent to RCB for peptides analysis by ESI-MS QUAD TOF. The mass profiling of the peptides was received in mascot format, which gave a probability bases protein score and included the likely hood that match is true. The identified proteins were compared by using the protein blast tool from NCBI database with their corresponding general information identifier number, accession number, and expected mass of protein (kDa). The result suggested 365 different proteins constituted ~50 kDa protein band in serum of women experiencing spontaneous pregnancy loss. Among the identified serum proteins, many serum proteins were previously reported by different researchers and matched. However, 34 proteins out of 365 were found to be still unnamed, unrecognized and their mechanism still unidentified (NCBI database accession number BAG35159,

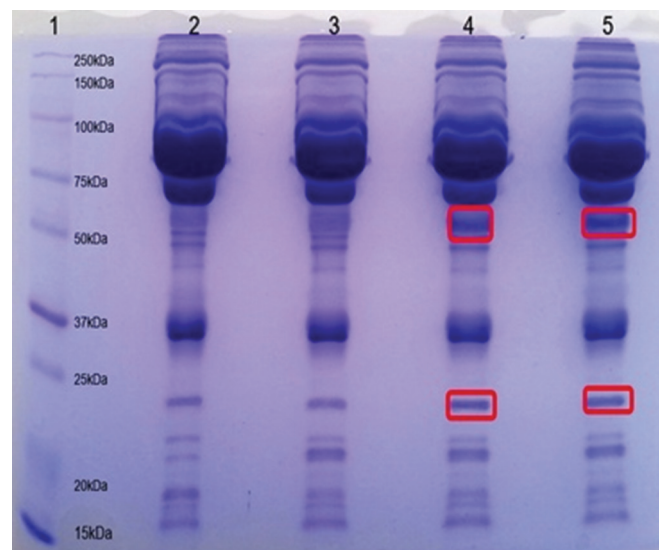


Figure 1: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) stained with Coomassie Brilliant Blue R-250 showing the variations in protein bands between controls and cases, where; Lane 1: Standard molecular weight Protein marker. Lane 2 and 3: Serum of normal pregnant women. Lane 4 and 5: Serum of women with spontaneous pregnancy loss

BAG60658, BAC04226, BAF82062, BAC85173, CAA28987, BAC0542, BAC85388, BAC85175, BAC05016, BAC04208, BAC85172, BAC85697, BAC85202, BAC85483, CAA32649, BAC86457, BAG62929, BAG62978, BAC85390, BAC87457, BAB55072, BAG62914, BAC85361, BAB71634, CAA28659, BAG62938, BAC85740, BAC86210, BAG36492, BAG61384, BAG56732, BAH14043, BAF82933) suggesting their probable role in mechanism of spontaneous pregnancy loss.

Further, the hormonal profile and serum total proteins were also compared in total, 1st and 2nd trimester participants among normal pregnant women and women experiencing pregnancy loss. The levels of TT₃, total proteins, β-hCG, and prolactin were found to be low, whereas TSH was high in women experiencing spontaneous pregnancy loss compared to normal pregnant women and the difference was statistically significant ($P \leq 0.05$). On the other hand, difference in TT₄ level among normal pregnant women and women experiencing spontaneous pregnancy loss was found to be statistically insignificant ($P \geq 0.05$) [Table 1].

In the 1st trimester participants, the results suggested TT₃, total proteins, and prolactin level were low, whereas, TSH level was high in women experiencing spontaneous pregnancy loss compared to normal pregnant women and the difference was statistically significant ($P \leq 0.05$).

However, difference in the level of β-hCG and TT₄ was found to be insignificant ($P \geq 0.05$) [Table 2].

In the 2nd trimester participants, TT₃, total proteins, β-hCG, and prolactin level were found to be low, whereas, TSH was high in women experiencing spontaneous pregnancy loss compared to normal pregnant women and the difference was statistically significant ($P \leq 0.05$). Further, TT₄ level between normal pregnant women and women experiencing spontaneous pregnancy loss was found to be statistically insignificant ($P \geq 0.05$) [Table 3].

Further, correlation analysis in total data of women experiencing spontaneous pregnancy loss was done and statistically significant inverse correlation was observed between TT₃ and TSH and β-hCG and TSH levels, where correlation coefficient was ($r = -0.336$), ($P = 0.009$) and ($r = -0.372$), ($P = 0.003$), respectively. However, nonsignificant correlations ($P \geq 0.05$) were observed between TT₃ and TT₄; TT₄ and TSH; PRL and β-hCG and PRL and TSH of women experiencing spontaneous pregnancy loss [Table 4 and Figures 2, 3]. Similar results were observed in the correlation analysis of 1st and 2nd trimester women experiencing spontaneous pregnancy loss in this study.

Further, in total data of normal pregnant women, statistically significant inverse correlation between TT₃ and TSH level was found [Figure 4], where correlation coefficient was ($r = -0.313$), ($P = 0.015$). However,

Table 1: Comparison of serum total protein, total tri-iodothyronine, total thyroxine, thyroid stimulating hormone, beta human chorionic gonadotropin and prolactin between complete/total data of normal pregnant women (controls) and women experiencing spontaneous pregnancy loss (cases) using Student's t-test

Parameter	Mean±SD		Mean difference	P	Result
	Controls (n=60)	Cases (n=60)			
TP (g/dL)	7.42±0.88	6.85±0.66	0.57	0.001	Significant
TT ₃ (ng/dL)	1.35±0.33	1.10±0.54	0.25	0.003	Significant
TT ₄ (µg/dL)	8.63±0.92	8.79±1.07	0.15	0.40	NS
TSH (µIU/mL)	2.48±0.95	5.59±3.21	3.11	0.001	Significant
β-hCG (mIU/mL)	42,211±15,090.32	35,820±11,617.82	6391.38	0.01	Significant
Prolactin (ng/mL)	52.89±18.44	38.38±11.04	14.50	0.001	Significant

TP: Total protein, TT₃: Total tri-iodothyronine, TT₄: Total thyroxine, TSH: Thyroid stimulating hormone, β-hCG: Beta human chorionic gonadotropin, NS: Nonsignificant, SD: Standard deviation

Table 2: Comparison of 1st trimester serum total protein, total tri-iodothyronine, total thyroxine, thyroid stimulating hormone, beta human chorionic gonadotropin and prolactin between normal pregnant women (controls) and women experiencing spontaneous pregnancy loss (cases) using Student's t-test

Parameter	Mean±SD		Mean difference	P	Result
	Controls (n=35)	Cases (n=35)			
TP (g/dL)	7.37±0.87	6.95±0.68	0.42	0.029	Significant
TT ₃ (ng/dL)	1.32±0.30	1.12±0.49	0.21	0.043	Significant
TT ₄ (µg/dL)	8.76±0.77	8.77±0.95	0.001	0.99	NS
TSH (µIU/mL)	2.41±0.93	5.19±3.01	2.78	0.001	Significant
β-hCG (mIU/mL)	38,516±14,053.19	34,980±10,147.75	3535.17	0.23	NS
Prolactin (ng/mL)	51.58±14.55	37.50±10.88	14.08	0.001	Significant

TP: Total protein, TT₃: Total tri-iodothyronine, TT₄: Total thyroxine, TSH: Thyroid stimulating hormone, β-hCG: Beta human chorionic gonadotropin, NS: Nonsignificant, SD: Standard deviation

Table 3: Comparison of 2nd trimester serum total protein, total tri-iodothyronine, total thyroxine, thyroid stimulating hormone, beta human chorionic gonadotropin and prolactin between normal pregnant women (controls) and women experiencing spontaneous pregnancy loss (cases) using Student's *t*-test

Parameter	Mean±SD		Mean difference	P	Result
	Controls (n=25)	Cases (n=25)			
TP (g/dL)	7.50±0.89	6.70±0.61	0.80	0.001	Significant
TT ₃ (ng/dL)	1.35±0.34	1.03±0.60	0.31	0.026	Significant
TT ₄ (µg/dL)	8.46±1.09	8.82±1.22	0.36	0.28	NS
TSH (µIU/mL)	2.59±0.97	6.16±3.46	3.56	0.001	Significant
β-hCG (mIU/mL)	47,384±15,239.40	36,994±13,544.82	10390.0	0.01	Significant
Prolactin (ng/mL)	54.71±13.03	39.62±11.37	15.09	0.005	Significant

TP: Total protein, TT₃: Total tri-iodothyronine, TT₄: Total thyroxine, TSH: Thyroid stimulating hormone, β-hCG: Beta human chorionic gonadotropin, NS: Nonsignificant, SD: Standard deviation

Table 4: Correlation analysis between the different hormones in women experiencing spontaneous pregnancy loss

Parameters (n=60)	r	P	Result
TT ₃ and TT ₄	-0.08	0.545	Correlation was not significant at $P \geq 0.05$
TT ₃ and TSH	-0.336	0.009	Significant inverse correlation $P=0.009$
TT ₄ and TSH	0.031	0.817	Correlation was not significant at $P \geq 0.05$
PRL and β-hCG	0.309	0.061	
PRL and TSH	-0.249	0.55	
β-hCG and TSH	-0.372	0.003	Significant inverse correlation $P=0.003$

TP: Total protein, TT₃: Total tri-iodothyronine, TT₄: Total thyroxine, TSH: Thyroid stimulating hormone, β-hCG: Beta human chorionic gonadotropin, PRL: Prolactin, NS: Nonsignificant

nonsignificant correlation was observed among TT₃ and TT₄; TT₄ and TSH; PRL and β-hCG; PRL and TSH; β-hCG and TSH of normal pregnant women ($P \geq 0.05$) [Table 5]. Similar correlation was observed in the results of 1st and 2nd trimesters of normal pregnant women in this study.

Discussion

Pregnancy is a complex biological process with large number of women observing no preexisting endocrine abnormalities, however, 8%–12% develop alterations in hormonal profile^[9] leading to miscarriage or spontaneous pregnancy loss.^[2] Many previous studies have suggested that these alterations in maternal hormonal profile during gestation might be due to the result of altered expression of proteins;^[10] because protein or peptides are the building block of most of the hormones and many important biological components. Even though pregnancy loss has been extensively studied and variations in proteins have been observed, but the exact mechanism responsible for this has not yet been fully understood.

In this study, variations in the serum protein bands resolved on 12% SDS-PAGE in the 1st trimester pregnancy were found approximately around 150 kDa, 50 kDa, and 25 kDa. The protein band of ~150 kDa was found to be highly expressed in normal pregnant women and was visualized as

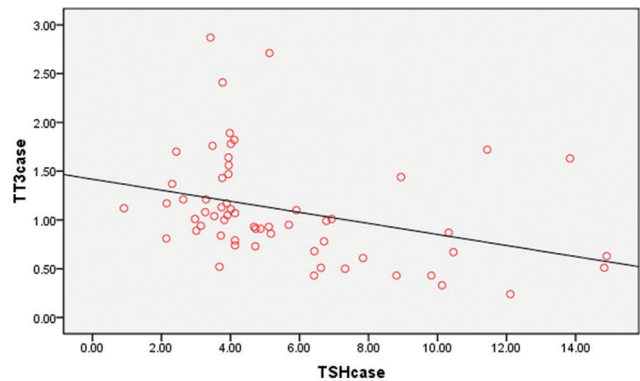


Figure 2: Scatter graph representing the inverse correlation between total tri-iodothyronine and thyroid stimulating hormone in total number of women experiencing spontaneous pregnancy loss. TT3: Total tri-iodothyronine, TSH: Thyroid stimulating hormone

strong dark band, while in women experiencing spontaneous pregnancy loss, this band was lightly expressed. On the other hand, in women experiencing spontaneous pregnancy loss, protein bands of ~50 kDa and ~25 kDa were found to be highly expressed and visualized as strong dark bands, while in normal pregnant women, these bands were lightly expressed [Figure 5]. In the sera of 2nd trimester participants, variations in protein bands were noted around ~50 kDa and ~25 kDa and visualized as strong dark bands in women experiencing spontaneous pregnancy loss, while in normal pregnant women, they seemed to be weakly expressed [Figure 6]. Further, the selected ~50 kDa protein band was analyzed by ESI-MS QUAD TOF and the mass profiling of the peptides in mascot format confirmed the findings that some proteins are differentially expressed in women with spontaneous pregnancy loss. Although this protein band of ~50 kDa was found to be of 365 different proteins and due to some resource restrictions, we could not carry 2-D electrophoresis for further separation. However, out of these 365 different proteins, 34 proteins were found to be unnamed and could be potential source for the diagnosis of high-risk pregnancy or to elucidate the mechanism of miscarriage. Similar to the result of this study, Cui *et al.*, also identified 78 differentially expressed serum proteins in women experiencing spontaneous

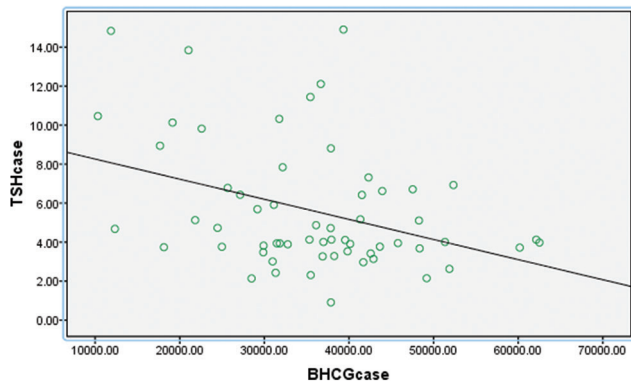


Figure 3: Scatter graph representing the inverse correlation between beta human chorionic gonadotropin and thyroid stimulating hormone in total number of women experiencing spontaneous pregnancy loss. TSH: Thyroid stimulating hormone, β -hCG: Beta human chorionic gonadotropin

pregnancy loss and suggested three different proteins that were closely related to pregnancy loss.^[11] Further, Wu *et al.* also demonstrated 151 proteins having significantly altered expressions among the normal pregnant women and women with recurrent spontaneous abortions.^[12] Another proteomic-based study using placental villous tissue identified 314 differently expressed proteins from recurrent miscarriage patients and these 314 proteins were found to play an important role in the growth of embryo.^[13] Thus, based on these observations and the results of this study, it is suggested that there are variations in the expression of different proteins between normal pregnant women and women experiencing spontaneous pregnancy loss, but unfortunately, none of these have been thoroughly studied and till date, none of these have been worked upon to be a specific biomarker for the identification of high-risk pregnancy or miscarriage. Since, villous tissue or follicular fluid is difficult to obtain, therefore, further research on these 34 unidentified proteins recognized to be differentially expressed in serum of women with spontaneous pregnancy loss may be an appropriate approach in finding specific biomarker for preventing pregnancy-related complications.

Further, to have better knowledge regarding serum total protein levels, comparison between normal pregnant women and women experiencing spontaneous pregnancy loss in total, 1st and 2nd trimester participants was done and the findings suggested statistically significant low values in women experiencing spontaneous pregnancy loss [Table 1]. This decrease in serum total protein concentration might be due to increase in fluid volume (hydremia) in women experiencing spontaneous pregnancy loss as compared to normal pregnant women.^[14] Since, to the best of our knowledge, this is the first study in this region, comparing serum total protein level between normal pregnant women and women experiencing spontaneous pregnancy loss, Thus, more research is suggested to validate these findings.

Pregnancy loss has been attributed to several factors, among all the important factors; thyroid hormones alterations are widely

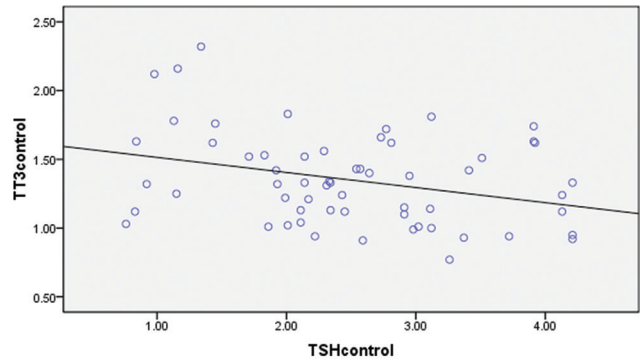


Figure 4: Scatter graph representing the inverse correlation between total tri-iodothyronine and thyroid stimulating hormone in total number of normal pregnant women. TT3: Total tri-iodothyronine, TSH: Thyroid stimulating hormone

Table 5: Correlation analysis between the different hormones of normal pregnant women

Parameters (n=60)	r	P	Result
TT ₃ and TT ₄	-0.045	0.734	Correlation was not significant at $P \geq 0.05$
TT ₃ and TSH	-0.313	0.015	Significant inverse correlation $P=0.015$
TT ₄ and TSH	-0.121	0.359	Correlation was not significant at $P \geq 0.05$
PRL and β -hCG	0.224	0.085	
PRL and TSH	-0.024	0.118	
β -hCG and TSH	-0.022	0.865	

TP: Total protein, TT₃: Total tri-iodothyronine, TT₄: Total thyroxin, TSH: Thyroid stimulating hormone, β -hCG: Beta human chorionic gonadotropin, PRL: Prolactin, NS: Nonsignificant

accepted. Hence, to check, whether the variation in the levels of thyroid hormones plays any role in frequency of miscarriages, mean serum TT₃ was compared between normal pregnant women and women experiencing spontaneous pregnancy loss in total, 1st trimester and 2nd trimester participants and the findings suggested that there was a statistically significant difference in the levels of TT₃ between normal pregnant women and women experiencing spontaneous pregnancy loss and the level of TT₃ was low in women experiencing spontaneous pregnancy loss [Tables 1-3]. This agreed with the finding of Ramandeep K *et al.*, (2017) which also suggested low TT₃ level in women experiencing spontaneous pregnancy loss as compared to normal pregnant women.^[8] Another study suggested that untreated hypothyroidism (subclinical or overt), at the time of conception is associated with higher miscarriage rate as compared to euthyroid subjects.^[15] On contrary to these finding, Gahlawat *et al.* have suggested nonsignificant difference in the level of TT₃ between normal pregnant women and women experiencing spontaneous pregnancy loss.^[16]

Similarly, the comparison of serum TT₄ in total, 1st and 2nd trimester participants suggested statistically nonsignificant difference between normal pregnant women and women experiencing spontaneous pregnancy loss [Tables 1-3]. In agreement to the result of current

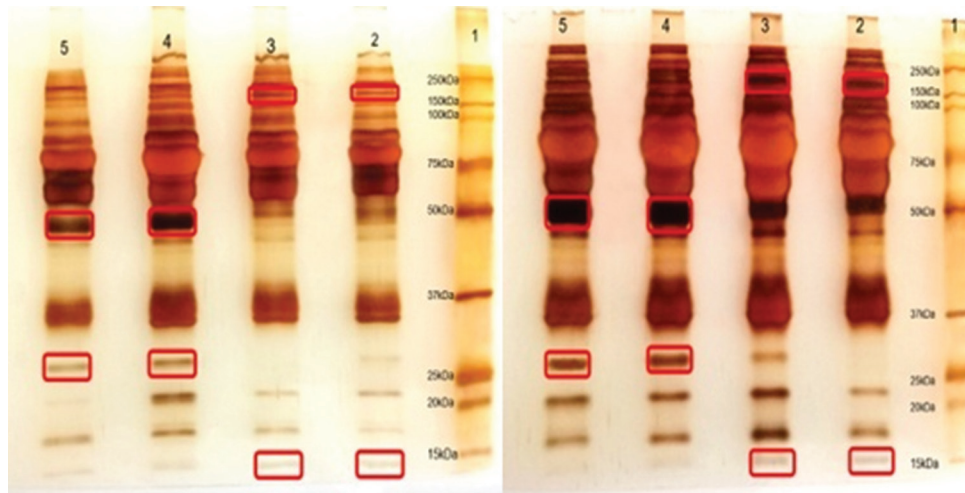


Figure 5: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) protein profile of normal pregnant women and women with spontaneous pregnancy loss in 1st trimester, where; Lane-1: Standard molecular weight protein marker, Lane 2 and 3: Serum of normal pregnant women of 1st trimester, Lane 4 and 5: Serum of women with spontaneous pregnancy loss of 1st trimester

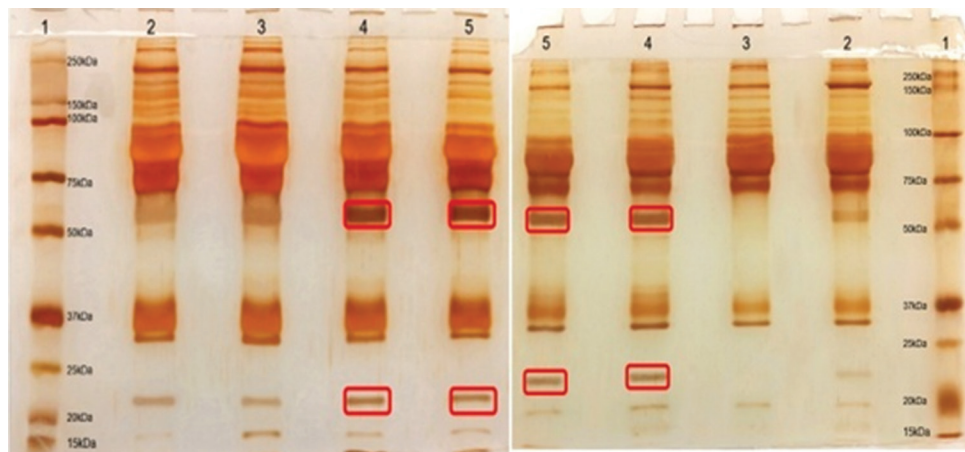


Figure 6: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) protein profile of normal pregnant women and women with spontaneous pregnancy loss in 2nd trimester, where; Lane-1: Standard molecular weight protein marker, Lane 2 and 3: Serum of normal pregnant women of 2nd trimester, Lane 4 and 5: Serum of women with spontaneous pregnancy loss of 2nd trimester

study, Ramandeep K *et al.*(2017)^[8] and Sreelatha *et al.*^[17] also suggested a similar observation. However, study by Donmez *et al.* and Rao *et al.* suggested low level of total T₄ in recurrent miscarriage as compared to normal pregnant women.^[18,19]

Further, comparison of mean serum TSH level among normal pregnant women and women experiencing spontaneous pregnancy loss in total, 1st and 2nd trimester participants suggested statistically significant high level of TSH in women experiencing spontaneous pregnancy loss [Tables 1-3]. In agreement to the current study, many previous studies had suggested that increased TSH without overt thyroid abnormality is one of the self-determining factors for higher number of pregnancy loss and with a higher frequency of stillbirth.^[20,21] The significant higher risk of early pregnancy loss in the present study may be due to the disturbance in thyroid hormones; as fetus is entirely dependent on transplacental passage of maternal thyroid

hormones, so any alteration in maternal thyroid profile is suggested to result in defective placental development and consequently spontaneous pregnancy loss. Hence, women with altered thyroid hormones are suggested to be monitored regularly to prevent the adverse pregnancy outcome.

The comparison of another important hormone, β-hCG suggested statistically significant difference in total and 2nd trimester normal pregnant women and women experiencing spontaneous pregnancy loss, while in the 1st trimester, the difference was statistically nonsignificant, although the mean levels were found to be low [Tables 1-3]. In agreement to this, Hamed *et al.* suggested that pregnant women with a history of miscarriage have significant low levels of β-hCG.^[22] Another study have also suggested that risk of miscarriage is much higher in women with low β-hCG and proposed low β-hCG level as a possible causative factor for miscarriage.^[23] This abnormal outcome

might be due to fundamental role of β -hCG in stimulating the corpus luteum to produce progesterone to maintain the pregnancy.^[24] On the other hand, Wang *et al.* suggested no association between the level of β -hCG and miscarriage.^[25] However, in the current study, the mean levels of β -hCG were low in both the trimester of women experiencing spontaneous pregnancy loss as compared to normal pregnant women, suggesting low β -hCG levels might be one of the possible reasons for miscarriage. The probable reasons for this association may be because of delay in trophoblast growth due to embryonic aneuploidy, immune or thrombophilic disturbances or inadequate secretion of β -hCG by fetoplacental unit which further result in inadequate progesterone production and may be the cause for embryonic death.

Similarly, the comparison of mean serum prolactin levels between normal pregnant women and women experiencing spontaneous pregnancy loss in total, 1st trimester and 2nd trimester participants suggested statistically significant low prolactin level in women experiencing spontaneous pregnancy loss as compared to normal pregnant women [Tables 1-3]. In agreement to the finding of this study, an increased risk of miscarriage has been shown to be associated with low basal serum prolactin concentration.^[26] Association of lower prolactin levels and miscarriage may be because of the fact that prolactin levels are responsible for down regulation of 20- α -hydroxysteroid dehydrogenase to allow progesterone levels to rise; therefore, insufficient prolactin is associated with insufficient production of progesterone, which is necessary to maintain the healthy pregnancy.^[27] In contrary, Gürbüz *et al.* suggested nonsignificant relationship between hyperprolactinemia and recurrent pregnancy loss and further suggested that low level of prolactin may be detrimental for pregnancy maintenance.^[28]

Thus, considering the results of above-mentioned hormones, this study suggests that there are variations in the concentration of different hormones in women experiencing spontaneous pregnancy loss. Therefore, regular monitoring should be done for different hormones on routine basis for pregnant women with high-risk pregnancy and women with a history of recurrent pregnancy loss.

On the other hand, most of the biomolecules in the human beings are interconnected as well as precisely controlled by the secretion and expression of each other, so altered expression of one biomolecule may lead to up or downregulation in the levels of others. Thus, considering the above statement, an attempt was made to identify association among TT_3 and TT_4 in women experiencing spontaneous pregnancy loss and normal pregnant women and the finding suggested no significant correlation in both [Tables 4 and 5]. The mechanism behind nonsignificant correlation between TT_3 and TT_4 in current study might be due to the expression of thyroxine binding globulin (TBG)

which is enhanced during pregnancy under the influence of estrogen. TBG has 20-fold greater affinity for T_4 , which may result in additional elevation in the levels of T_4 compared to T_3 .^[29] On contrary to the results of the current study, Harada *et al.* suggested positive correlation between serum TT_3 and TT_4 levels in both normal pregnant women and women experiencing spontaneous pregnancy loss.^[30]

On the other hand, a significant inverse correlation was observed between TT_3 and TSH in women experiencing spontaneous pregnancy loss and normal pregnant women [Tables 4, 5 and Figures 2, 4] which might be due to the thyroidal adjustment, which is regulated primarily through the normal pituitary-thyroid feedback mechanisms, i.e. by TSH stimulation of the thyroid gland. In agreement to the finding of the current study, Gahlawat *et al.* also suggested significant inverse correlation between T_3 and TSH.^[16] Further, a nonsignificant correlation was observed between TT_4 and TSH in women experiencing spontaneous pregnancy loss and normal pregnant women [Tables 4 and 5]. The nonsignificant correlation among TT_4 and TSH may be due to high estrogen levels during pregnancy, which lead to increase in serum T_4 -binding globulin, further independently increasing serum TT_4 without any elevation in TSH level.^[31] In contrary to this, study of Gahlawat *et al.* suggested significant negative correlation between T_4 and TSH in both normal pregnant women and women experiencing spontaneous pregnancy loss.^[16]

Prolactin and β -hCG are suggested to be the important hormones for normal pregnancy; however, no statistically significant correlation was observed in the study [Tables 4 and 5]. Probably, they are vital to maintain the pregnancy, but not in a synergistic manner. Moreover, till now, no study has reported any correlation between the two hormones, suggesting way out to carry more research in this direction. Similarly, nonsignificant correlation between prolactin and TSH observed in both normal pregnant women and women experiencing spontaneous pregnancy loss [Tables 4 and 5], which is contrary to the findings of earlier studies which suggested significant positive correlation between TSH and prolactin levels in normal pregnant women^[32] or in the infertile subjects.^[32,33] Further, significant inverse correlation between β -hCG and TSH in women experiencing spontaneous pregnancy loss [Figure 3] and nonsignificant correlation in normal pregnant women [Tables 4 and 5] in the present study emphasized the thyroid stimulating activity of β -hCG for the possible mechanism of inverse correlation.^[34] Benhadi *et al.* also suggested the direct relation between TSH and pregnancy loss was regardless of β -hCG levels.^[23]

Hence, results of this study suggest that there are variations in the expression of serum protein levels aligned with the alterations in serum hormonal levels. Since proteins are the constituents of many hormones, therefore, association in

alteration of two in parallel to each other is well understood. Moreover, alteration in serum protein levels and findings of 34 unnamed proteins in women with spontaneous pregnancy loss are the significant observations of this study, which may explore the path for the diagnosis of high-risk pregnancy. Further, isolation and identification of these 34 unidentified proteins can throw some light on alteration of hormones in spontaneous pregnancy loss and its pathophysiology as well as can help in the development of specific serum biomarkers for the diagnosis of high-risk pregnancies.

The major limitations of the study were the identification of proteins on the basis of only 1-D electrophoresis and peptide identification only from one selected protein band. Therefore, further studies are warranted in this direction for isolation and specific identification of individual proteins of stated protein bands for better interpretation in the outcomes of pregnancy.

Conclusion

This study indicated differential expression of proteins as well as association between the alteration of serum proteins and serum hormonal profile in women experiencing spontaneous pregnancy loss. Therefore, screening for serum protein variations along with serum hormonal levels may provide useful lead about the fate of high-risk pregnancy. Thus, the development of precise biomarker based on altered protein profile may enable the clinicians to target administration of potential therapies in early stages to women with high-risk pregnancy.

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Conflicts of interest

There are no conflicts of interest.

References

- Wang Y, Jiang X, Jia L, Wu X, Wu H, Wang Y, *et al.* A single-cell characterization of human post-implantation embryos cultured *in vitro* delineates morphogenesis in primary syncytialization. *Front Cell Dev Biol* 2022;10:835445.
- Smith ML, Schust DJ. Endocrinology and recurrent early pregnancy loss. *Semin Reprod Med* 2011;29:482-90.
- Malaza N, Masete M, Adam S, Dias S, Nyawo T, Pheiffer C. A systematic review to compare adverse pregnancy outcomes in women with pregestational diabetes and gestational diabetes. *Int J Environ Res Public Health* 2022;19:10846.
- Kamble G, Bhattacharya BM. Miscarriage and associated risk factors in India: A brief review. *MOJ Womens Health* 2017;4:84-6.
- Punches BE, Johnson KD, Acquavita SP, Felblinger DM, Gillespie GL. Patient perspectives of pregnancy loss in the emergency department. *Int Emerg Nurs* 2019;43:61-6.
- Dugas C, Slane VH. Miscarriage. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2018.
- Dunn D, Turner C. Hypothyroidism in women. *Nurs Womens Health* 2016;20:93-8.
- Ramandeep K, Kapil G, Harkiran K. Correlation of enhanced oxidative stress with altered thyroid profile: Probable role in spontaneous abortion. *Int J Appl Basic Med Res* 2017;7:20-5.
- Kaur R, Gupta K. Endocrine dysfunction and recurrent spontaneous abortion: An overview. *Int J Appl Basic Med Res* 2016;6:79-83.
- Iglesias-Platas I, Martin-Trujillo A, Petazzi P, Guillaumet-Adkins A, Esteller M, Monk D. Altered expression of the imprinted transcription factor PLAGL1 deregulates a network of genes in the human IUGR placenta. *Hum Mol Genet* 2014;23:6275-85.
- Cui Y, He L, Yang CY, Ye Q. iTRAQ and PRM-based quantitative proteomics in early recurrent spontaneous abortion: Biomarkers discovery. *Clin Proteomics* 2019;16:36.
- Wu Y, He J, Guo C, Zhang Y, Yang W, Xin M, *et al.* Serum biomarker analysis in patients with recurrent spontaneous abortion. *Mol Med Rep* 2017;16:2367-78.
- Pan HT, Ding HG, Fang M, Yu B, Cheng Y, Tan YJ, *et al.* Proteomics and bioinformatics analysis of altered protein expression in the placental villous tissue from early recurrent miscarriage patients. *Placenta* 2018;61:1-10.
- Hartland AJ, Smith JM, Dunne F. Correcting serum fructosamine concentration for total protein or albumin concentration is not appropriate during Asian pregnancy. *Clin Chim Acta* 2000;292:175-80.
- Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A, Levalle O. Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid* 2002;12:63-8.
- Gahlawat P, Singh A, Nanda S, Kharb S. Thyroid dysfunction in early pregnancy and spontaneous abortion. *Biomed Biotechnol Res J* 2017;1:81-4.
- Sreelatha S, Nadagoudar S, Asha DL. The study of maternal and fetal outcome in pregnant women with thyroid disorders. *Int J Reprod Contracept Obstetr Gynecol* 2017;6:3507-14.
- Donmez M, Şişli T, Atuş A, Aydın Y. Spontaneous abortion and thyroid functions. *Perinat J* 2005;13:110-3.
- Rao VR, Lakshmi A, Sadhmani MD. Prevalence of hypothyroidism in recurrent pregnancy loss in first trimester. *Indian J Med Sci* 2008;62:357-61.
- Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, *et al.* Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 2011;21:1081-125.
- Murmu AK, Kumari S, Sharan A, Baskey S. Correlation of hypothyroidism with pregnancy outcome in preeclampsia. *OSR JDMS* 2018;17:72-8.
- Hamed M, Dawakhly H, Shaaban A, Ismail S. Predictive value of the triplemarkers: Serum Progesterone, Ca 125 And Beta-hcg. The possible occurrence of miscarriage. *Am J Res Commun* 2016;4:47-62.
- Benhadi N, Wiersinga WM, Reitsma JB, Vrijkotte TG, Bonsel GJ. Higher maternal TSH levels in pregnancy are associated with increased risk for miscarriage, fetal or neonatal death. *Eur J Endocrinol* 2009;160:985-91.
- Montagnana M, Trenti T, Aloe R, Cervellini G, Lippi G. Human chorionic gonadotropin in pregnancy diagnostics. *Clin Chim Acta* 2011;412:1515-20.
- Wang A, Kort J, Westphal L. Miscarriage history association with euploid embryo transfer outcomes. *Reprod Biomed Online* 2019;39:617-23.

26. Shah PB, Gupta K, Bedi M. Comparative study on different hormones between normal pregnant women and women experiencing miscarriage. *Int J Appl Basic Med Res* 2020;10:240-4.
27. Parker VJ. Hypothalamic Mechanisms Mediating Inhibition of Prolactin Secretion Following Stress in Early Pregnant Mice; 2012.
28. Gürbüz B, Yalti S, Fiçicioğlu C, Ozden S, Yildirim G, Sayar C. Basal hormone levels in women with recurrent pregnancy loss. *Gynecol Endocrinol* 2003;17:317-21.
29. Refetoff S. Abnormal thyroid hormone transport. In: *Endotext*. South Dartmouth (MA): MDText.com, Inc.; 2015.
30. Harada A, Hershman JM, Reed AW, Braunstein GD, Dignam WJ, Derzko C, *et al.* Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *J Clin Endocrinol Metab* 1979;48:793-7.
31. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, *et al.* 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* 2017;27:315-89.
32. Bassey IE, Udoh AE, Essien OE, Isong IK, Gali RM, Archibong EE. Thyroid hormones and prolactin levels in infertile women in Southern Nigeria. *J Clin Diagn Res* 2015;9:C13-5.
33. Sushilendu V, Singh K, Kumari R. To evaluate the level of serum prolactin, FSH and LH in females with thyroid disorders. *Indian J Clin Biochem* 2022;33:S81.
34. Zgliczynska M, Ostrowska M, Szymusik I, Ciebiera M, Kosinska-Kaczynska K. Maternal thyroid function in multiple pregnancies – A systematic review. *Frontiers in Endocrinology* 2022;13.