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Original Article

The Link between Impaired Complement Factor H and Dysregulation of Immunity in Recurrent Spontaneous Abortion

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Abstract

Background: Recurrent spontaneous abortion (RSA) affects 1–2% of couples, yet nearly half of the cases remain unexplained. Dysregulation of the complement system, particularly impaired activity of complement factor H (CFH), may play a key role, but its contribution to early pregnancy loss is not well defined.
Objective: This case-control study investigated the role of CFH alterations in women with first-trimester RSA and assessed its potential as a biomarker for RSA risk assessment.
Methods: The study investigated CFH changes among 55 pregnant women from Sulaymaniyah, Iraq, including 35 with RSA and 20 with normal pregnancies (NP). Serum CFH concentrations were measured using ELISA, and its mRNA expression was quantified by RT-qPCR. Exon 9 (rs1061170) polymorphisms were then analyzed by Sanger sequencing.
Results: RSA cases exhibited significantly reduced CFH protein levels (median 150.5 vs. 174.7 ng/mL; $p = 0.0118$, $\eta^2 = 0.125$, power = 82.4%) and decreased CFH gene expression ($p = 0.0187$, $\eta^2 = 0.099$, power = 64.3%), both independent of maternal age, weight, and BMI. Receiver operating characteristic analysis demonstrated moderate discriminatory accuracy for both protein (AUC = 0.71) and mRNA expression (AUC = 0.69). Exploratory sequencing revealed the presence of the rs1061170 C allele in RSA cases, although genotype frequencies were not significantly different.
Conclusions: These findings provide novel evidence that quantitative and genetic alterations in CFH may contribute to impaired complement regulation at the maternal–fetal interface and predispose women to first-trimester RSA. CFH shows potential as part of a multi-marker biomarker panel for RSA risk assessment, warranting validation in larger and longitudinal studies.

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1. Introduction:

Recurrent spontaneous abortion (RSA) is defined as the loss of two or more pregnancies before 20 weeks of gestation (1, 2), affecting about 1 to 2% of couples. It remains one of the most challenging complications in reproductive medicine (3). Despite progress in diagnostics, around half of RSA cases have no explanation, indicating the presence of underlying immunological complications (4). Most abortion cases (50-80%) occur in the 1st trimester, which highlights the importance of early molecular and immunological studies (5, 6).

The complement system, a critical component of innate immunity, has been implicated as a

major player in maintaining pregnancy and counteracting pregnancy complications. The cascade participates in implantation, vascular remodelling and apoptotic cell clearance (7, 8). Among its three pathways, the alternative pathway acts as an efficient amplification loop and is active on a constitutive basis through spontaneous hydrolysis of C3 (9, 10). Whilst this basal activity facilitates immune surveillance, any loss of control or regulation can lead to deposition of excessive C3b and tissue damage (11, 12).

Complement factor H (CFH) is the primary soluble regulator of the alternative pathway. Under normal conditions, it blocks C3 convertase (C3bBb) generation and promotes the degradation of C3b, thus preventing extensive cascade amplification (13-15).

During pregnancy, CFH is synthesized by the liver as well as by trophoblasts and decidual tissues, where it constructs a protective barrier against complement-mediated injuries on the maternal–fetal interface (16, 17). In addition, CFH interacts with molecules on apoptotic and necrotic cells (e.g., DNA, annexin II, histones), thereby suppressing secondary inflammatory reactions (17, 18).

When CFH levels are decreased, this protective balance is lost, leading to excess C3a/C3b deposition, which results in consequent inflammation and injury of the trophoblast (13, 19). Debris and apoptotic cells shed by the placenta further fuel complement activation (19, 20). The resulting immune imbalance impairs placental development and maternal–fetal tolerance (21-23), providing a putative mechanistic association between low CFH levels and increased pregnancy loss risk in RSA (19, 24-26).

Beyond pregnancy, atypical hemolytic uremic syndrome, C3 glomerulopathy, and age-related macular degeneration are among the systemic conditions linked to CFH dysregulation or deficiency (27, 28). A reduction in CFH activity in reproductive contexts has been linked to preeclampsia and compromised maternal-fetal tolerance (29-31). However, to date, few studies have addressed the specific role of CFH in first-trimester RSA.

In addition to quantitative differences, genetic variation could also affect the function of CFH. This has been seen in the common CFH exon 9 variant rs1061170 (c.1204C>T; p.His402Tyr, often termed Y402H in previous literature) that affects binding affinity for heparin and C-reactive protein (27). Despite the strong association with macular degeneration, this polymorphism has also been associated with pregnancy outcomes (32). However, its association with RSA is still not well known and warrants further study.

Given that approximately 50% of RSA cases remain unexplained and CFH has not been systematically studied in this context, particularly in Middle Eastern populations, we aimed to fill this gap by investigating whether quantitative and genetic alterations in CFH contribute to first-trimester RSA in Iraqi women. We assessed serum CFH levels, gene expression in peripheral blood and performed an exploratory sequencing of exon 9 polymorphisms among first-trimester RSA. By combining these data, we sought to elucidate whether defective regulation of the alternative pathway by CFH plays a role in early pregnancy loss.

2. Materials and Methods:

This experimental, applied study was cross-sectional and conducted on 75 women in the first trimester of their pregnancy who were referred to hospitals in Sulaymaniyah, Kurdistan Region of Iraq. Gestational status was based on serum β-hCG levels and transvaginal ultrasonography when needed. After inclusion/exclusion criteria, 55 women were included: 35 with RSA and 20 with NP. Women with a high risk or known history of diabetes, autoimmune diseases, smoking and alcohol addiction, thalassemia, increased level of D-dimer or abnormal thyroid-stimulating hormone (TSH) in the blood and recent acute

infectious and severe systemic diseases affecting immunity were excluded according to their medical history and physical examination. Demographic information, such as age, weight, and body mass index (BMI), was collected using standardised questionnaires. The exclusion/inclusion process is shown in Table 1.

Table 1: Participant Enrollment and Selection Process

Group	Total Enrolled (N)	First Trimester (N)	Excluded – Second Trimester (N)	Excluded – Other Reasons (N)	Final Selected (N)
RSA	42	35	0	7	35
NP	33	20	9	4	20
Total	75	55	9	11	55

***** RSA = recurrent spontaneous abortion; NP = normal pregnancy; N = number of samples. Of 75 women initially enrolled, only those in the first trimester were included. Excluded cases comprise second-trimester pregnancies or those with other exclusion criteria (e.g., systemic illness, incomplete data).

2.1 Sample Collection and Processing

Between November 2024 and March 2025, blood samples were collected. Aliquots of whole blood and serum were separated and stored at –80°C until further use. While genomic DNA was isolated as needed, total RNA from whole blood was extracted immediately upon collection. A spectrophotometer (EzDrop 1000, Blue-Ray Biotech, Taiwan) was used to measure the concentration and purity. Both were subsequently stored at –80°C.

2.2 Measurement of Serum CFH Levels

Serum CFH concentration was quantified using human-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech; Cat. No. SL3852Hu) from Hangzhou, China, according to the manufacturer's guidelines. Standards and samples were applied to 96-well plates pre-coated with specific antibodies, and absorbance was read at 450 nm using a Chromate® ELISA microplate reader (Awareness Technology Inc., USA). Protein concentrations were calculated from standard curves with the assay detection limit of 0.1 ng/mL. In total, ELISA measurements were obtained from 50 serum samples, comprising 20 from the NP group and 30 from the RSA group.

2.3 Gene Expression Analysis by RT-qPCR

Total RNA was isolated from whole blood using the QIAamp RNA Blood Mini Kits (Qiagen, Germany; Cat. No. 52304). Specifically designed primer sets (Sigma-Aldrich, Germany; Table 2) and qPCRBIO SYGreen 1-Step Go Lo-ROX reagent (PCR Biosystems, UK) were used following the manufacturer's instructions to quantify the samples. Amplification reactions were conducted on a Bio-Rad CFX96 real-time PCR detection system. Relative mRNA expression levels were calculated using the delta quantification cycle (ΔCq) method, with GAPDH as the reference gene. GAPDH stability was verified across groups. The difference between the Cq of the target gene and the internal control gene GAPDH is called ΔCq, which is inversely proportional to the gene expression level. Additionally, to provide a biological perspective and visual

representation of relative expression differences, 2^(-ΔΔCq) was calculated using NP group as the calibrator group. Statistical analyses, however, were performed solely on ΔCq values, with the 2^(-ΔΔCq) plot included for biological interpretation only.

To ensure comparability, the RT-qPCR assays were carried out on the same samples previously analyzed by ELISA. Additionally, five RSA blood samples with hemolyzed serum (unsuitable for ELISA protein quantification) but intact RNA extracted from blood were included to strengthen statistical power. Altogether, 55 samples were examined, comprising 20 NP and 35 RSA cases.

2.4 Exon 9 Sequencing and SNP Detection

DNA was extracted from the blood samples using the AddPrep Genomic DNA Extraction Kit from AddBio (South Korea) according to the manufacturer's instructions. The extracted DNA samples were then subjected to polymerase chain reaction (PCR) directed at a target region in exon 9 of the CFH gene. This analysis was focused explicitly on the rs1061170 polymorphism, as its functional involvement in CFH regulation is well documented. Primers against this region were designed expressly by this study using the NCBI Primer-BLAST (Table 2) and synthesized by Macrogen Inc. (Seoul, South Korea;). The PCR was carried out in a TurboCycler thermal cycler (Blue-Ray Biotech, Taiwan) using the AddStart Taq Master Mix (AddBio Inc., Korea) under optimized cycling conditions specific to the primer pair.

PCR products were analysed on 1.5% agarose gels, and band specificity was verified by visualisation under UV light with a Bio-Rad Gel Documentation System. A 100 bp DNA ladder and safe dye (Gene Direx, Taiwan) were selected as a DNA molecular weight marker and visualization standard.

A subset of 9 DNA samples (4 from the NP group and 5 from the RSA group) was selected for sequencing. This subset was analysed in an exploratory manner to determine if the exon 9 polymorphism rs1061170 of CFH could be identified and possibly examined in more extensive genetic research to decide whether or not it could be linked to RSA. Amplicons were submitted to Macrogen Inc. (Seoul, South Korea) and purified for Sanger sequencing in the forward direction.

Chromatograms were examined using Finch TV software, and sequences were compared to the human CFH reference sequence (NCBI RefSeq: NG_007259.1, assembly GRCh38/hg38). SNP identities were verified against the dbSNP database, and variant positions were further examined using the UCSC Genome Browser and NCBI Variation Viewer. The Human Genome Variation Society nomenclature was used for the descriptions of the variants.

Table 2: Primers Used For RT-qPCR, Conventional PCR, and Sequencing Analysis

Target Gene	Primer 5′→3′	Product Size-bp	Tm °C	Reference sequence
CFH In RT-qPCR	F- 5′ CACCTCAGATAGAACACGGAAC 3 R- 5′ CTGAAACCACCCTCACAAGTAT 3′	100 bp	60°C	NM_000186.4
CFH In Conventional PCR	F- 5′AGGGTTTCTTCTTGAAAATCACAGG 3′ R-5′AGTGTACTTACTGACACGGATG 3′	479 bp	60°C	NG_007259.1

2.5 Statistical Analyses

Statistical analyses and figures were produced using GraphPad Prism 9.0. The normality of continuous variables was tested using the Shapiro–Wilk test. As the data were not distributed normally, comparisons between groups regarding demographic variables (age, weight, height, BMI), serum CFH levels and its ΔCq values were performed with a Mann–Whitney U. To better understand whether demographic factors had an independent effect on CFH expression, exploratory multiple linear regression models were performed including age, weight and BMI as covariates and then, Correlations of unadjusted clinical characteristics and immune markers were assessed by Spearman test. Through using Receiver Operating Characteristic (ROC) curve analyses, the diagnostic performance of CFH protein and gene expression was investigated. The results were presented as the area under the curve (AUC) and associated 95% confidence intervals (CI). G*Power version 3.1.9 was used to perform post hoc power analyses in order to evaluate the sensitivity of group difference detection. Lastly, to examine the distribution between genotypes across groups, Fisher's exact test was applied.

Levels of statistical significance were designated as:

- Non-significant (p-values > 0.05)
- Significant ($p \leq 0.05$)
- Very significant ($p \leq 0.01$)
- Highly significant ($p \leq 0.001$)

Effect sizes (η^2) were calculated via web-based computing program (Lenhard and Lenhard, 2022), for between-groups assessment of the effect size of observed differences. eta squared (η^2) values were defined as: large (≥ 0.14), medium ($\eta^2 = 0.06$–0.13), small ($\eta^2 = 0.01$–0.05), and negligible ($\eta^2 = 0$-0.009).

2.6 Ethical approval:

This study was approved by the Ethics Committee of the College of Medicine, University of Sulaimani, Kurdistan Region, Iraq (Approval No. 343; Meeting No. 25, dated October 23, 2024). All participants provided written informed consent before enrollment. Personal data were collected confidentially, with strict attention to privacy, autonomy, and minimization of potential harm, in accordance with institutional ethical standards.

3. Results:

3.1 Serum Level of Complement Factor H

First, we investigated the serum CFH levels in the groups that had normal pregnancy (NP) and recurrent spontaneous abortion (RSA). As seen in Fig. 1, the RSA group's levels were statistically significantly lower than those of the NP group. This is indicated by the ELISA results (Mann–Whitney U = 174, p = 0.0118). Serum levels of the RSA group were, on average, 17.8 ng/mL lower than the NP group, based on the Hodges-Lehmann estimate. This difference was validated by descriptive statistics. The mean for the RSA group was 149.7 ± 11.7 ng/mL (95% CI: 145.3–154.0), whereas the mean for the NP group was 166.0 ± 21.7 ng/mL (95% CI: 155.8–176.1). A mean rank comparison verified this downward shift (RSA = 21.3 vs. NP = 31.8). The 82.4% post hoc power analysis indicates sufficient sensitivity to identify the observed difference.

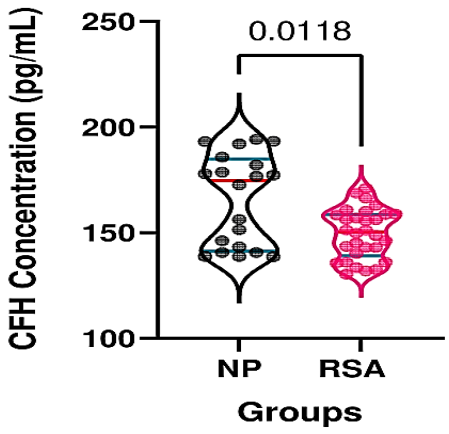

Fig. 1. Serum concentrations of CFH in NP and RSA

groups. Data are non-normally distributed and presented as medians (red bar) with interquartile ranges (IQR; blue bar). Mann-Whitney U tests were used for group comparisons. Effect size was calculated using eta-squared (η^2). The RSA group (n = 30) showed a significant decrease in serum CFH protein concentrations compared to the NP group (n = 20), with a medium $\eta^2 = 0.125$. The exact *p*-value was 0.0118. NP = normal pregnancy; RSA = recurrent spontaneous abortion.

3.1.1 Multiple linear regression analysis for protein concentrations

After controlling for age, weight, and BMI, exploratory multiple linear regression analyses were conducted on CFH protein to determine whether the elevated protein concentrations in the RSA group were unrelated to demographic variables. The analysis showed that the protein levels were not significantly predicted by age (p = 0.253), weight (p = 0.350), or BMI (p = 0.944). Since the model only explained 11.9% of the variance ($R^2 = 0.119$) and was not statistically significant overall (p = 0.210), these parameters had no effect on CFH concentrations. Fig. 2 displays the plot.

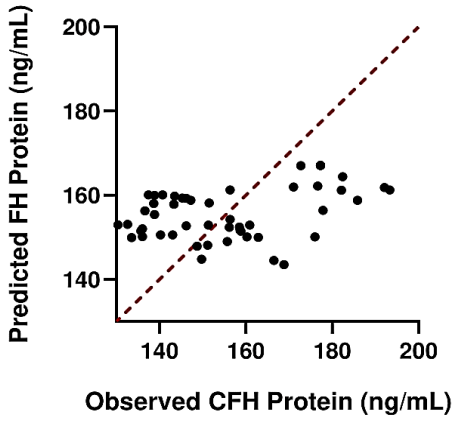

Fig. 2. Multiple linear regression plot of observed versus predicted CFH serum concentrations. Predictors included group (RSA vs. NP), age, weight, and BMI. The red dashed line represents the line of perfect prediction (y = x). None of the predictors were significant (all > 0.05; $R^2 = 0.119$). Each dot represents an individual participant. R^2 = coefficient of determination; NP = normal pregnancy; RSA = recurrent spontaneous abortion.

3.2 Gene Expression Analysis of CFH

Following protein analysis, we used RT-qPCR to assess CFH gene expression to determine whether changes in peripheral blood gene expression were consistent with changes in serum levels. The ΔCq values from RT-qPCR analysis of RNA samples were used to measure the levels of mRNA expression; lower values denote higher expression levels. The median-IQR range is used to display the ΔCq data for the RSA and NP. The RT-qPCR results showed a significant difference in CFH mRNA expression between the RSA and NP groups (Mann–Whitney U = 216.5, p = 0.0187), with a medium effect size ($\eta^2 = 0.099$). In RSA (n = 35), the median ΔCq values were 3.950 (IQR: 3.820–4.300), while in NP (n = 20), they were 3.845 (IQR: 3.608–3.983). The Hodges-Lehmann estimate of the shift was 0.180, while the actual difference between medians was 0.105. However, it is important to note that the post hoc power for detecting this significant difference was 64.3%, which falls below the conventional 80% threshold. This moderate statistical power indicates a limitation in the sensitivity of our study to detect the observed effect. Fig. 3 illustrates this finding with boxplots that show the RSA group's downward shift.

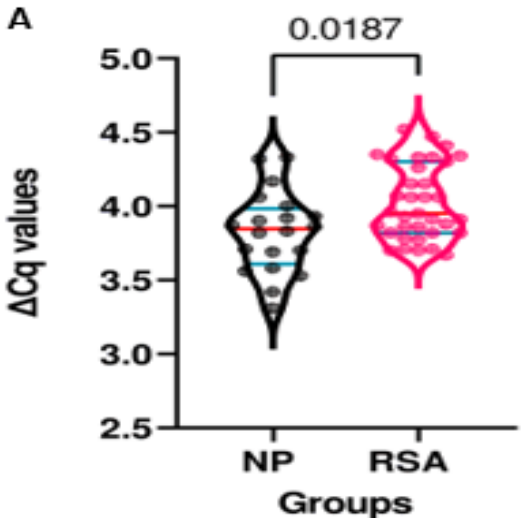

Fig. 3. Gene expression analysis based on ΔCq values. (A) mRNA expression of *CFH* in RSA and NP groups: Bars represent median ± IQR of ΔCq values. Normality

was not confirmed; therefore, group comparisons were performed using the Mann–Whitney U test. mRNA expression was significantly reduced in RSA compared with NP (U = 216.5, p = 0.0187, $\eta^2 = 0.099$), as reflected by higher ΔCq values.

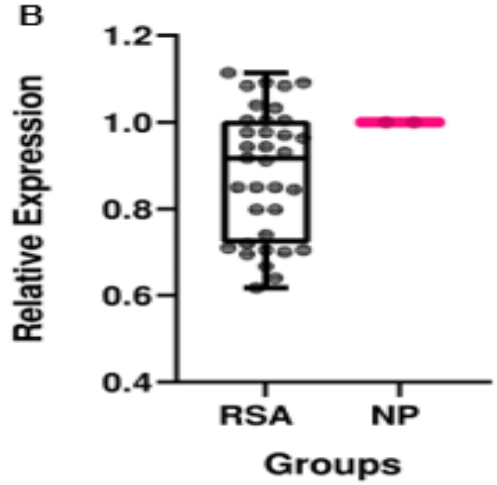

Fig. 3. Gene expression analysis based on ΔCq values. (B) Relative expression of the *CFH* gene using the 2^(-ΔΔCq) method. ΔΔCq values were calculated as (ΔCq_RSA – mean ΔCq_NP). NP samples were set to a fold-change of 1 as the calibrator, and 2^(-ΔΔCq) was computed to show expression relative to NP. This panel illustrates the magnitude of CFH mRNA downregulation in RSA. NP = Normal Pregnancy; RSA = Recurrent Spontaneous Abortion; SEM = Standard Error of the Mean.

3.2.1 Multiple linear regression analysis for mRNA expression

We also used multiple linear regression analysis for this section to ascertain whether demographic factors had an impact on CFH expression. CFH ΔCq values were subjected to multiple linear regression models with age, weight, and BMI as covariates.

Multiple linear regression analysis revealed that group status (RSA vs. NP) was a significant independent predictor of gene expression for CFH ΔCq (β = 0.247, 95% CI: 0.085–0.409, p = 0.0034; Fig. 4). The total model explained 18.8% of the variance (R^2 = 0.188) and was statistically significant (p = 0.0316). On the other hand, BMI (p = 0.217), weight (p = 0.792), and age (p = 0.857) were not significant covariates.

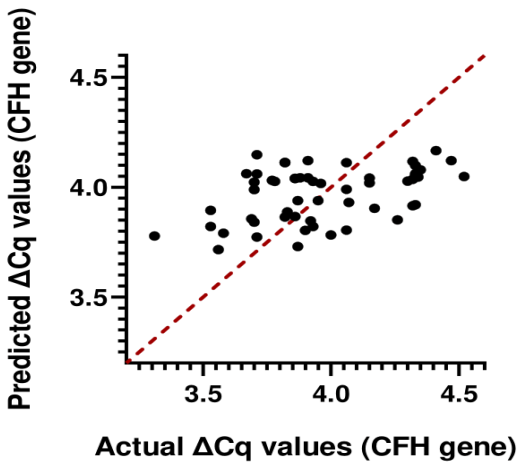

Fig. 4. Scatter plots for predicted versus actual ΔCq values for CFH gene expression. Predictors in the regression model included group (NP vs. RSA), age, weight, and BMI. The red dashed line represents y = x (perfect prediction). The model had moderate predictive accuracy (R^2 = 0.188) and identified group status (RSA vs. NP) as a significant independent predictor (p = 0.0034), while age, weight, and BMI were not significant predictors (all p > 0.05). R^2 = coefficient of determination; NP = normal pregnancy; RSA = recurrent spontaneous abortion.

3.3 Correlation of Demographic Variables with CFH Protein and Gene Expression

In addition to regression analysis, correlation analyses were conducted to investigate the unadjusted strength and direction of the associations between the immune marker (CFH) in the RSA group and each of the clinical characteristics (age, weight, and BMI) individually. The findings demonstrated no significant relationship between either group's CFH gene expression or protein concentrations and age, weight, or BMI (all r values were between –0.15 and 0.15, p > 0.05).

3.4 ROC-Based Evaluation of CFH as a Biomarker

We evaluated the potential diagnostic value of CFH as a biomarker after examining its relationship to clinical variables. To do this, we performed Receiver Operating Characteristic (ROC) curve analyses to determine how well the protein and mRNA levels could distinguish between the RSA and NP groups. Fig. 5

displays the corresponding ROC curves from analyses conducted on a complete dataset devoid of missing values. Protein levels and ΔCq values showed moderate diagnostic accuracy, according to the analyses. The protein levels had an area under the curve (AUC) of 0.71 (95% CI: 0.549–0.871, p = 0.0126; RSA n = 30, NP n = 20), and the ΔCq values had an AUC of 0.69 (95% CI: 0.544–0.837, p = 0.0195; RSA n = 35, NP n = 20).

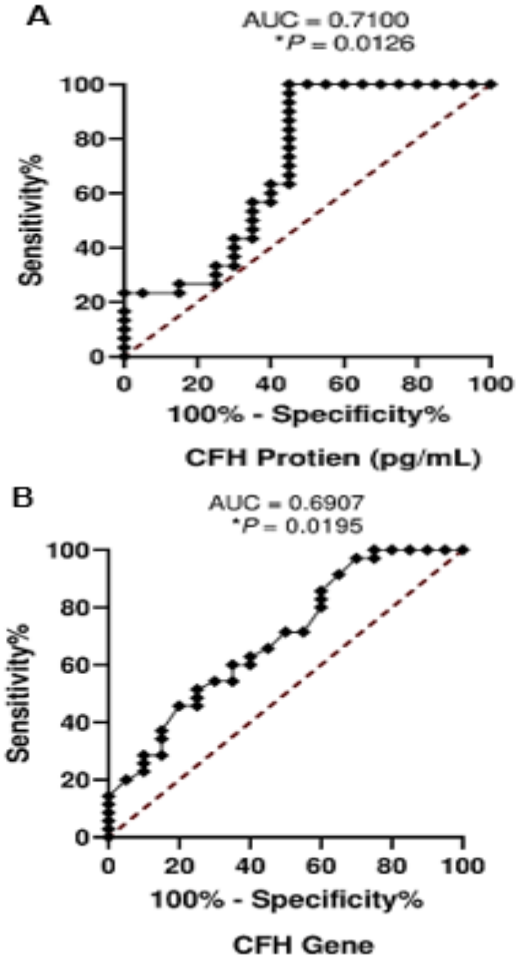

Fig. 5. Receiver Operating Characteristic (ROC) curves for CFH protein and mRNA expression levels. (A) Protein-level analysis was conducted on (n = 30; RSA) and (n =20; NP) samples. (B) mRNA expression analyses were based on ΔCq values from (n = 35; RSA) and (n = 20; NP) samples. The farther the distance of the curve from the red dashed line, the better the discriminatory power of the test. The AUCs and p-values are shown above each plot. AUC = Area Under the Curve; RSA = Recurrent Spontaneous Abortion; NP = Normal Pregnancy.

3.5 Genotypic Distribution of CFH rs1061170

Lastly, we looked into the possibility that RSA was linked to genetic variation at exon 9 (rs1061170). In both RSA and NP samples, PCR amplification of exon 9 produced a product of the anticipated size (479 bp). Fig.6 shows representative gel electrophoresis results, which confirm that the target fragment was amplified successfully and specifically without contamination or nonspecific bands. This confirmed the amplicons' quality before sequencing.

Table III summarizes the distribution of CFH rs1061170 (His402Tyr, C>T) genotypes. Every member of the NP group (n=4) had homozygosity for the T allele (T/T, 100 %). Two women were heterozygous C/T (40 %) and three were T/T (60 %) in the RSA group (n = 5), as shown in (Fig.7). There were no C/C genotypes found in either group. The C-allele was only found in the RSA group (0.20), whereas the T-allele frequency was 1.00 in NP and 0.80 in RSA. The genotype distributions of NP and RSA did not differ statistically significantly, according to Fisher's exact test (p = 0.467, two-tailed). The formula for allele frequencies is (2×TT + 1×CT) / (2×n). To determine whether genetic variation and quantitative deficiency may work in tandem, these genotypic data were further examined in relation to CFH protein and transcript levels in the Discussion.

Table 3: Distribution of CFH rs1061170 (His402Tyr; C>T) Genotypes in Study Groups

Group	N	C/C	C/T	T/T	T-allele freq.	C-allele freq.
NP	4	0 (0 %)	0 (0 %)	4 (100 %)	1.00	0.00

RSA	5	0 (0 %)	2 (40 %)	3 (60 %)	0.80	0.20
Total	9	0 (0 %)	2 (22.2 %)	7 (77.8 %)	0.89	0.11

***** Coordinates reported on GRCh38; dbSNP: rs1061170. Reference allele C encodes His402; alternate allele T encodes Tyr402. NP = normal pregnancy; RSA = recurrent spontaneous abortion; N = Number of samples.

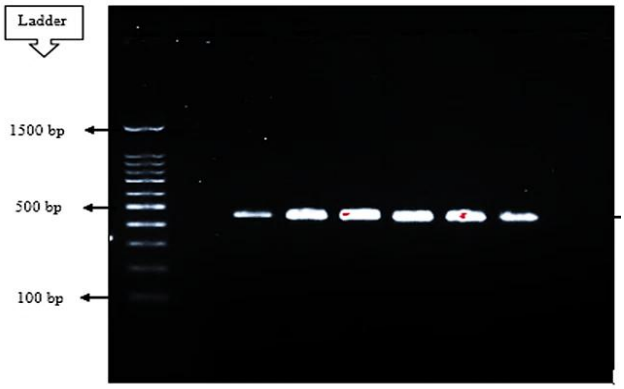

Fig. 6. Agarose gel electrophoresis of PCR products for CFH exon 9 amplification. CFH-specific primers were used to create representative PCR amplicons (479 bp) from the genomic DNA of NP and RSA samples. Lane M: 100 bp DNA ladder; Lane 1: no-template control (NTC; reaction with primers and master mix but no DNA); Lanes 2–7: PCR products from a few study samples exhibiting the predicted band size. Following staining with a safe dye, bands were visible under UV light.

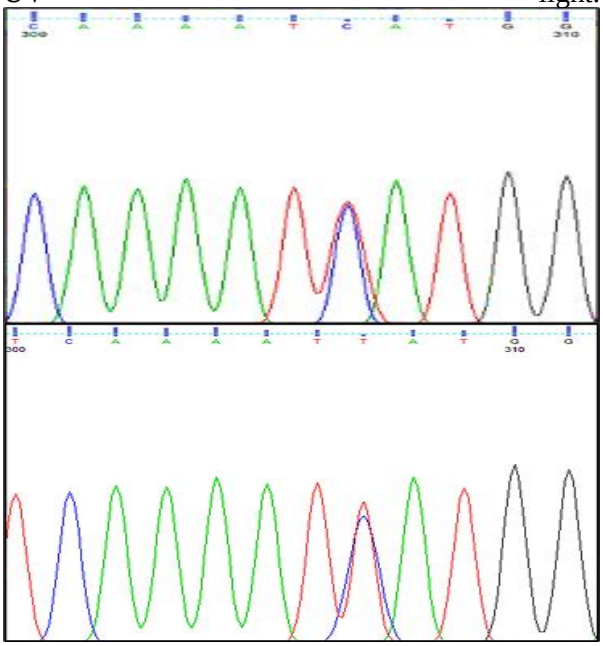

Fig. 7. Forward-strand Sanger chromatograms (Finch TV), showing heterozygotes (C/T) with double peaks at the SNP position. SNP = Single Nucleotide Polymorphism.

4. Discussion:

This case-control study provided new evidence on the involvement of human CFH in RSA among Iraqi women, by incorporating protein, gene expression, and explorative sequencing assay. We observed a significant decrease in the protein levels ($p = 0.0118$, $\eta^2 = 0.125$, power = 82.4%). Since only a few studies have examined CFH in the context of RSA, especially in the first trimester, this observation is considered novel. In contrast, in a cohort study measuring maternal CFH level in pregnant women, no significant differences were found between miscarriage and normal pregnancy (33). On the other hand, a Colombian prospective cohort study revealed that women who had lower levels of CFH in the first trimester were more likely to have preterm birth (34). This aligns with our findings and implies that a lack of this protein could lead to a variety of unfavourable pregnancy outcomes.

Regarding CFH mRNA, the majority of earlier research has concentrated on placental tissue and complications other than RSA. As an instance, decreased CFH mRNA expression in the placenta tissue has been demonstrated in patients having preeclampsia (17). Our study extends these findings by examining peripheral blood mRNA expression. To increase statistical power, five additional RSA samples with hemolyzed serum but intact RNA were included in the RT-qPCR analysis. We were able to show that mRNA expression of CFH was significantly downregulated in peripheral blood in RSA as compared to normal pregnancy ($p = 0.0187$), the post hoc power for gene expression was 64.3%. While this is below the conventional 80% threshold, the observed medium effect size ($\eta^2 = 0.099$) suggests a substantial biological association

between its mRNA downregulation and RSA. This indicates that the lack of higher power is a function of sample size rather than a lack of biological relevance, emphasizing the need for independent validation in larger cohorts to confirm these systemic trends.

Regression analysis demonstrated that these variations in serum CFH and its mRNA expression were not affected by maternal age, weight, or body mass index (BMI). The results indicate that CFH dysregulation in RSA is predominantly immunologically driven rather than demographically influenced. Although these analyses were exploratory due to the non-normal data distribution, both regression and correlation analyses consistently indicated that the variables were not significant predictors of CFH expression levels. In contrast to this, in pregnant women with gestational diabetes mellitus, researchers found correlations between CFH and age and BMI (35). We propose that the impact of metabolic factors might be condition-specific rather than RSA-generalizable.

ROC analyses showed moderate discriminatory accuracy (AUC = 0.71 and 0.69, respectively). While these values demonstrate that CFH is a potential biological indicator of RSA, they fall short of the thresholds required for independent clinical diagnosis. Therefore, CFH may be more effectively utilized as a component within a multi-marker predictive panel rather than a standalone diagnostic tool.

In addition to quantitative reductions in CFH, genetic variation rs1061170 at chr1: g.196690069C>T may further compromise CFH function. Previous studies reported that the Y402H polymorphism influences both CFH serum levels and binding affinity (36). Together with our finding of decreased CFH expression, we hypothesise that variant-related functional effects may compound to lower CFH availability.

Both the RSA and NP groups had the rs1061170 (H402Y) polymorphism identified in our exploratory sequencing analysis. In the RSA group, there were two women with C/T and three with T/T, whereas all of the women in the NP group were homozygous for the T allele (T/T), with no C/C genotypes. Notably, all of our sequenced samples consistently carried the T allele at this position, while the NCBI-aligned CFH reference genome sequence carries a C allele. This pattern suggests that T is the major (more frequent) allele in this specific population, while the C allele (as C/T heterozygotes) acts as the minor, risk-associated variant.

Statistical analysis revealed no significant difference in genotype distribution between groups (Fisher's exact test, $p = 0.467$). The exploratory sequencing was performed on a very small subset of samples ($n = 9$; 4 NP, 5 RSA); therfore, the lack of statistically significant differences should be interpreted cautiously. Despite this, the presence of the C allele only in RSA cases may indicate a context-dependent role, where the C allele's known functional deficiency (i.e., poor binding to heparin and CRP) could be relevant in the pathological environment of RSA.

Taken together with the observed quantitative reduction in CFH protein and mRNA, the presence of the C allele in RSA cases may contribute to a 'double-hit' effect. Both genetic variation and lower CFH levels could synergistically compromise complement regulation at the maternal–fetal interface, although this is still speculative and requires confirmation in larger studies. Importantly, the observed quantitative reductions in CFH protein and mRNA remain statistically significant independent of these preliminary genetic observations.

Our results are broadly compatible with other studies, even though the sample size was small. C/T genotypes were reported to be more common in Korean women who experienced repeated pregnancy loss (32), and the C/C genotype has also been reported to increase the risk of recurrent early pregnancy loss in

comparison to sporadic early pregnancy loss (37). In terms of the Iraqi population, a study showed that the C/C genotype was linked to a higher risk of recurrent aphthous stomatitis, with C/T also playing a role (38). These highlights the novelty of our study in iraqi women and the need for further research in Middle Eastern populations.

There is ongoing discussion regarding the rs1061170 SNP's functional role. Because of its poor binding to heparin and CRP, the C allele (His402) is generally considered the risk variant (27, 39). However, population- and disease-specific effects have also been reported. Both the C/C and C/T genotypes are reliably linked to a higher risk of AMD (40, 41). In healthy people, the C/C genotype was associated with higher CRP and changed immune cell profiles (42). Notably, a meta-analysis study showed that the C/C genotype linked to AMD was not found to be associated with coronary heart disease (43). When combined, these results imply that both genetic background and disease context influence allele-related susceptibility at rs1061170.

Conclusion:

This study offers new evidence linking significantly lower CFH protein concentrations and gene expression to RSA in first-trimester women. These differences were independent of maternal age, weight, and BMI. However, the medium effect size and limited statistical power for the CFH mRNA analysis (64.3%) indicate that these transcriptional differences require confirmation in larger studies before firm conclusions can be drawn. These results suggest that pregnancy loss could be associated with an imbalance in the alternative complement pathway's regulation. While the exploratory sequencing of exon 9 did not identify any statistically significant differences in the distribution of rs1061170 (H402Y) genotypes, C/T heterozygotes were detected only among RSA cases, with all other samples being T/T homozygous. It is possible that genetic variation may interact with reduced CFH availability further to compromise complement regulation at the maternal–fetal interface.

Collectively, the findings are consistent with a "double-hit" hypothesis where combined quantitative insufficiency and qualitative genetic variation in CFH may contribute to complement overactivity and trophoblast damage in RSA. Although CFH alone demonstrated only moderate discriminatory ability (AUC = 0.71 and 0.69), it might have potential as part of a future multi-marker panel. However, its potential utility remains preliminary and must be confirmed in future studies with larger sample sizes, bidirectional sequencing, and longitudinal follow-up to clarify the relationship between CFH variants and adverse pregnancy outcomes.

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Conflict of interest: The authors declare that there are no conflicts of interest related to this work.

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