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Does Complement Factor I Correlate with Complement Component 4b in HIV Infected Patients with Preeclampsia?

Kompleman Faktör I HIV ile Enfekte Preeklampside Kompleman Bileşen 4b ile İlişkili Midir?

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Abstract

Objective: This study aims to determine the concentration of complement factor I (CFI) and complement component 4b (C4b) in normotensive (N) pregnant women compared to women with preeclampsia (PE) and human immunodeficiency virus (HIV).

Materials and Methods: The study population consisted of preeclamptic (n=38) and normotensive (n=38) pregnant women, who were further stratified by HIV status into HIV-positive (n=19) and HIV-negative (n=19) groups. Serum levels of CFI and C4b were determined using the Bio-Plex multiplex immunoassays. Data analysis was conducted using Stata (version 12) and Graphpad Prism (8.0.1).

Results: A statistically significant downregulation of C4b was noted in the N+ve vs. N-ve, PE+ve in comparison with the N+ve group (p=0.0001) and in PE+ve compared to the PE-ve pregnancies. CFI levels were significantly upregulated in the N+ve vs. N-ve groups (p=0.004) and downregulated in PE+ve compared to N+ve and in PE+ve vs. PE-ve groups (p=0.004). Significant positive associations were noted for C4b with parity, between the PE-ve and N+ve groups (r=0.47; p=0.04), and for CFI in the PE+ve group (r=0.68; p=0.0001). Negative significant associations were noted between C4b and systolic blood pressure (r=-0.53, p=0.01) and C4b and diastolic blood pressure (r=-0.78, p=0.0001). CFI also showed negative association with parity (r=-0.48, p=0.03).

Conclusion: This innovative study demonstrates a decline of C4b and CFI levels in PE+ve, compared to N+ve pregnancies. It is plausible that the downregulation may be attributed to complement mediated virolysis emanating from C3 convertase dysregulation in PE comorbidity with HIV infection.

Keywords: Preeclampsia, HIV, C4b, complement factor I

Öz

Amaç: Bu çalışmada amaç, insan immün yetmezlik virüsü (HIV) ile enfekte olan preeklamptik kadınlara kıyasla, normotansif (N) hamile kadınlarda kompleman faktör I (CFI) ve kompleman bileşeni 4b (C4b) konsantrasyonlarını belirlemektedir.

Gereç ve Yöntem: Çalışma popülasyonu preeklamptik (n=38) ve normotansif (n=38) hamile kadınlardan oluşmaktadır ve HIV durumlarına göre HIV-pozitif (n=19) ve HIV-negatif (n=19) gruplarına ayrıldı. Serum CFI ve C4b seviyeleri, Bio-Plex multipleks immünolojik testleri kullanılarak belirlendi. Veri analizi, Stata (versiyon 12) ve Graphpad Prism (8.0.1) kullanılarak yapıldı.

Bulgular: HIV+ ve HIV- arasında, N+ve grup ile kıyaslandığında PE+ve, PE-ve ile kıyaslandığında PE+ gruplarında istatistiksel olarak anlamlı bir C4b down regülasyonu gözlendi (p=0.0001). CFI seviyeleri N+ve vs. N-ve gruplarında anlamlı şekilde up-regüle (p=0.004), HIV+ ile karşılaştırıldığında PE+ve grubunda, PE+ve vs. PE-ve gruplarında down regüle idi (p=0.004). Pariteli C4b için, PE-ve ve HIV+ hastalar arasında (r=0.47; p=0.04) ve CFI için PE+ve grubunda (r=0.68; p=0.0001) anlamlı pozitif ilişkiler kaydedildi (r=0.68; p=0.0001). C4b ile sistolik kan basıncı (r=-0.53, p=0.01) ve C4b ile diyastolik kan basıncı (r=-0.78, p=0.0001) arasında negatif anlamlı ilişkiler kaydedildi. CFI ayrıca parite ile negatif bağıntı saptandı (r=-0.48, p=0.03).

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Sonuç: Bu yenilikçi çalışma, N+ve gebeliklere kıyasla PE+ve'de C4b ve CFI düzeylerinde bir düşüş olduğunu göstermektedir. Bu az gen ifadesinin, HIV enfeksiyonu ile PE komorbiditesinde C3 konvertaz disregülasyonundan kaynaklanan kompleman aracılı virolize bağlı olarak gelişmiş olması olasıdır.

Anahtar Kelimeler: Preeklampsi, HIV, C4b, kompleman faktör I

Introduction

Human immunodeficiency virus (HIV) infects approximately 13% (8.2 million) of the South African (SA) population (1). Moreover, the KwaZulu-Natal province in SA, where 25% of women at their reproductive ages (15-49 years) are infected (1), is considered the epicenter of the HIV global pandemic (2). In KwaZulu-Natal Province in SA, tuberculosis co-infected with HIV infection, obstetric hemorrhage and hypertensive disorders of pregnancy account for 31%, 24% and 17% of maternal deaths, respectively (1,3). Approximately 83% of deaths due to hypertensive disorders of pregnancy emanate from preeclampsia (PE) development (3).

Preeclampsia is a pregnancy condition characterized by new onset of hypertension (≥140 mmHg systolic and/or ≥90 mmHg diastolic taken on two occasions at least 4 hours apart) accompanied by multi-organ involvement at or after 20 weeks of gestation (4). In SA, the prevalence of PE in primigravida is 12% (5). Preeclampsia is stratified, according to gestational age, as early onset PE (EOPE) if symptoms present between 20 and 33 weeks plus 6 days, and late onset PE at or after 34 weeks (4). The EOPE type is normally associated with defective placentation, placental lesions and adverse maternal and fetal outcomes (6,7). Trophoblast invasion is deficient with an absence of myometrial spiral artery remodeling and accompanied by an exacerbated immune response (8). This creates an ischemic and anti-angiogenic micro-environment (8). Notably, there is a neutralization of the exaggerated immune response in PE during HIV infection (9,10). However, the administration and use of antiretroviral therapy (ART) such as highly active antiretroviral therapy (HAART) restores immune response with resultant increased susceptibility to PE development (9-11).

The complement system is an integral yet fundamental portion of our innate immune response, which participates in pathogen opsonization, its elimination and the clearance of immune complexes (12). Complement components 4b (C4b) and complement factor I (CFI) are proteins integral to the activation and amplification of the immune response (12). Complement component 4b is the larger fragment of the central protein C4 that is vital for the classical and lectin pathway. Importantly, C4b covalently attaches to transformed self-tissue and pathogens, acting as an opsonin and marking the surface for removal (13). Furthermore, C4b is responsible for the building of active convertases that facilitate downstream initiation, via cleavage of complement C3 and C5 (14). A twofold decline in C4b has been previously reported in EOPE compared to normotensive pregnant women (15). However, this manifestation may be dysregulated in HIV infection and with ARV usage.

In the complement system, C4b interaction is regulated by CFI, a serine protease that mediates the cleavage of complement C3b and C4b (16-18). Hence, a dysregulation of CFI, also known as C3b/C4b inactivator, may increase complement activity and allow C4b to participate in the formation of C3 convertases (17). Additionally, this dysregulation may result in a continuous release of fluid-phase and cell-surface deposited C3b, via the alternative pathway, thereby depleting C3 levels (17,18). In the complement cascade, interaction between complement receptor 1 (CR1) and CFI allows the cleavage and inactivation of C3b and C4b, both of which directly interact with HIV membrane proteins gp120 and gp41 (19). HIV-bound C3b and C4b are inactivated during this process, signifying that CFI may attenuate HIV amplification (20). This study evaluates the concentration of serum C4b and CFI in the duality of HIV infection during PE, using the Bio-Plex immunoassay technology.

Materials and Methods

Ethical Approval

Institutional ethical approval (approval number: BCA338/17) and informed consent from all patients were obtained. Women were recruited at the antenatal clinic of a large urban regional hospital in Durban, South Africa.

Study Population

This study used archived serum samples to determine the circulating levels of C4b and CFI. Study groups (n=76) consisted of healthy normotensive pregnant women (n=38) and those diagnosed with PE (n=38). PE was defined as the onset of high blood pressure ≥140/90 mmHg and proteinuria >300 mg/dL after the 20th week of gestation (4). Both groups were further stratified by HIV status into normotensive HIV-positive (N+ve; n=19), normotensive HIV-negative (N-ve; n=19), preeclamptic HIV-positive (PE+ve; n=19) and preeclamptic HIV-negative (PE-ve; n=19) pregnant women. All HIV-positive women whether initially diagnosed or not or those who had discontinued drug treatment received HAART at the first antenatal visit. Exclusion criteria for the PE group was the presence of

chronic diabetes, chorioamnionitis, pre-existing seizure disorders, eclampsia, chronic hypertension, intrauterine fetal death, abruption placenta, pre-gestational diabetes, systemic lupus erythematous, chronic renal disease, sickle cell disease, thyroid disease, cardiac disease, and active asthma. Moreover, those with unknown HIV status were also excluded from the study.

ProcartaPlex™ Multiplex Immunoassay Method

The concentration of C4b and CFI was measured in all blood samples using the MILLIPLEX Human Complement Panel 1 - Immunology Multiplex Assay (Millipore by Sigma-Aldrich, catalog no: HCMP1MAG-19K). A 1:4 dilution series was used as standard. The C4b and CFI capture antibody-coupled magnetic beads were added to the plates and washed. Incubation with Standards, samples and blanks was performed for 2 hours at 20-25°C and then washed. Subsequently, a biotinylated detection antibody was allowed to incubate for 1 hour at 20-25°C. The plate was washed and the reporter dye (streptavidin-phycoerythrin) was added, and it was incubated for 1 hour. A final washing was performed and the beads were resuspended in assay buffer. The median fluorescence intensity was detected using the Bio-Plex®MAGPIXTM Multiplex Reader (Bio-Rad Laboratories Inc., USA) and the Bio-Plex ManagerTM software version 4.1.

Statistical Analysis

Study data were analyzed using STATA (version 12, STATACORP) and Graph Pad Prism version 8 (California, United States of America). Data were non-parametrically distributed using the D'Agostino test. The Kruskal-Wallis test and the Dunn's Post-hoc test were used to determine the significant difference between the groups. The Spearman's correlation was used to assess the relationship between C4b and CFI in the groups, and between clinical factors. Statistical significance was considered at *p<0.05, **p<0.01 and ***p<0.001.

Results

Clinical Characteristics

The clinical and biochemical characteristics are presented in Table 1. A significant difference was observed for maternal age (p=0.03), parity (p=0.01), gravidity (p=0.04), maternal weight (p=0.01), body mass index (BMI) (p=0.04), age of gestation (p=0.0001), systolic blood pressure (SBP) (p=0.0001) and diastolic blood pressure (DBP) (p=0.0001), C4b (p=0.0001) and CFI (p=0.004), respectively. Both the systolic and DBPs were significantly higher in PE compared to normotensive pregnancies (p<0.0001). At delivery, the gestational age was significantly lower in PE versus normal pregnancies (p<0.0001).

Circulating Levels of C4b and CFI

The circulating levels of C4b and CFI are shown in Figure 1A, B. The Kruskal-Wallis test revealed a statistically significant difference for circulating C4b levels between N+ve and N-ve (p=0.0001), PE-ve and N+ve (p=0.0001), PE+ve and N+ve (p=0.0001), and PE+ve and PE-ve (p=0.0001) groups, respectively (Figure 1A). Likewise, a significant difference was noted for CFI between N+ve and N-ve (p=0.0001), PE-ve and N-ve (p=0.0001), PE+ve and N+ve (p=0.0001), and PE+ve and PE-ve (p=0.0001) groups, respectively (Figure 1B).

Spearman's Correlation Between Circulating Levels of C4b, CFI and Clinical Factors

A bivariate Spearman's correlation test was used to analyze the relationship between circulating concentration of C4b and CFI and demographic/clinical factors in all groups. A positive correlation was observed for C4b between PE-ve and HIV+ve groups (r=0.47; p=0.04). Notably, CFI in the PE-ve group was positively associated with C4b in the PE+ve group (r=0.68; p=0.01).

Table 1. Clinical characteristics [n=76; median (25^{th}-75^{th} percentile)].

Maternal age (years)	Normotensive (n=38)		Pre-eclamptic (n=38)		p-value
	HIV-negative (n=19)	HIV-positive (n=19)	HIV-negative (n=19)	HIV-positive (n=19)	
	25.00 (20.00-29.00)*	31.00 (26.00-37.00)*	29.00 (19.00-35.00)	34.00 (24.00-38.00)*	0.03*
Parity	1.00 (0.00-1.00)*	2.00 (1.00-2.00)*	1.00 (0.00-2.00)*	2.00 (1.00-2.00)*	0.01**
Gravidity	2.00 (1.00-3.00)	3.00 (2.00-4.00)	2.00 (1.00-3.00)	3.00 (2.00-4.00)	0.04*
Maternal weight (kg)	74.00 (62.00-85.00)*	81.00 (66.00-94.00)	90.00 (69.00-115.00)*	79.50 (65.00-100.00)	0.01**
BMI	30.86 (25.39-35.04)*	32.05 (29.81-39.69)	37.04 (27.07-45.93)*	32.39 (25.82-36.89)	0.04*
Gestational age (weeks)	38.00 (37.00-38.00)*	37.00 (37.00-38.00)*	30.00 (28.00-33.00)*	30.00 (28.00-32.00)*	0.0001***
Systolic BP (mmHg)	113.00 (106.00-120.00)*	109.00 (103.00-114.00) *	154.00 (149.00-165.00)*	157.00 (145.00-168.00) *	0.0001***
Diastolic BP (mmHg)	72.00 (67-77)*	73.00 (64.00-77.00)*	102.00 (93.00-109.00)*	99.00 (97.00-106.00)*	0.0001***

BMI: Body mass index, BP: Blood pressure, HIV: Human immunodeficiency virus, PE: Preeclampsia, Statistical significance; *p<0.05, **p<0.01 and ***p<0.001

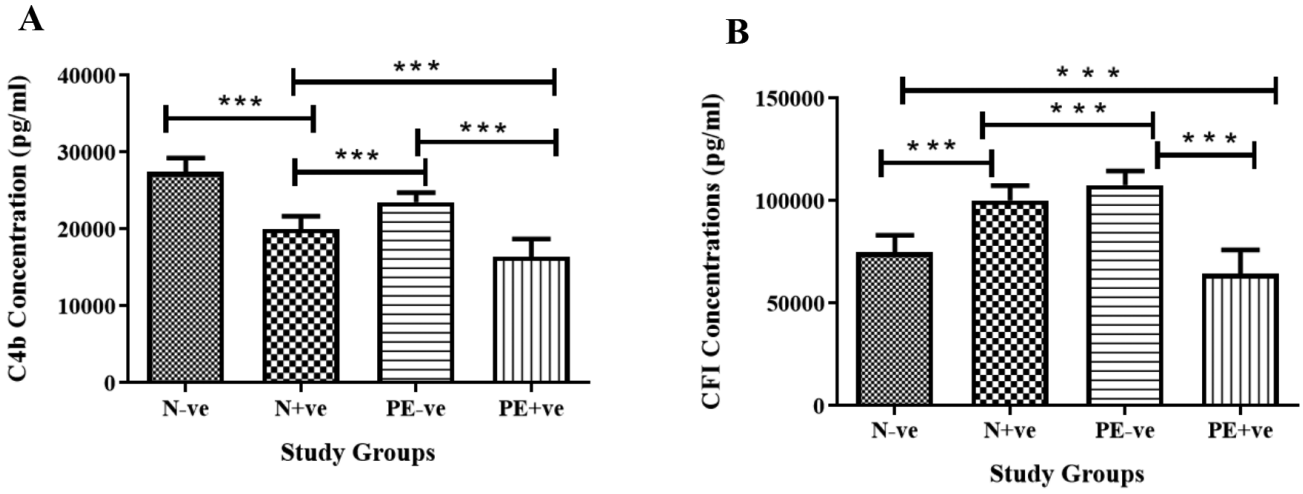

Figure 1. Serum concentrations (A-B, median and interquartile range) of (A) C4b (pg/mL) and (B) CFI (pg/mL) in HIV-negative normotensive pregnant (N-ve); HIV-positive pregnant (N+ve); HIV-negative preeclamptic (PE-ve) and HIV-positive preeclamptic (PE+ve) women. ***: p<0.0001 is considered statistically significant.

C4b: Component 4b, CFI: Complement factor I, HIV: Human immunodeficiency virus, PE: Preeclampsia

In the PE-ve group, a significant negative association was noted between C4b and SBP (r=-0.53, p=0.01) and between C4b and DBP (r=-0.78; p=0.0001). Similarly, in the PE-ve group, a significant negative association was noted between CFI and parity (r=-0.48; p=0.03). In contrast, a significant positive association was demonstrated between parity and C4b in the PE+ve group (r=0.55, p=0.01). No significant associations were noted for all other comparisons.

Discussion

This study reports a significant elevation in CFI level in the PE-ve group in comparison to the normotensive HIV-ve and HIV+ve groups. The up-regulation of CFI in the PE group may be linked to placental damage and release of antiangiogenic factors (21). Recently, an elevation of placental sFlt-1 was noted to be associated with complement activation (22). Our findings are in line with previous reports of similar elevated systemic and placental complement activation in PE (15,23,24). However, the CFI concentration in the PE+ve group was significantly lower than in the PE-ve group, possibly reflecting the effect of antiretroviral use.

We also reported a downregulation of C4b in PE+ve vs the HIV+ve group, suggestive of both antiangiogenic and antiretroviral effect. Previous studies have reported genetic polymorphisms in regulatory and complement genes, which predisposes PE development (21,25,26). Therefore, it is possible that gene mutations of C4b, CFI and/or regulatory proteins and/or aberrant receptor-ligand expression may underly their dysregulation in our study. Notably, haploinsufficiency arising from a CFI I398L mutation has been previously linked with CFI dysregulation in 18% of pregnant woman with PE (21). Regal et al. (23), in 2015, also observed a loss in homeostatic complement function emanating from mutations of complement gene regulators, contributing to increased complement activation. Additionally, a twofold elevation of C4 deficiency occurs in PE compared to normotensive conditions (27).

We reported significant up-regulation of CFI between the N+ve and N-ve pregnancies, which is corroborated by Wu et al. (28). Interestingly, Wu et al. (28), in 2017, identified that C3b and C4b complement proteins were directly interactive with both gp120 and gp41, HIV envelope glycoproteins that enhance viral propagation. CFI has an essential function of CFI to split and incapacitate C3b and C4b into constituents that would promote HIV suppression (20). In our study, the elevated CFI in the HIV+ group may emanate from this de-activation of the HIV/C3b/C4b complex (29). However, virions protect themselves from complement facilitated breakdown by using gp120 and gp41, to fuse factor H (30).

The CFI downregulation in PE+ve vs. PE-ve groups and in PE+ve compared to the HIV+ve groups corroborates previous *in vitro* studies that indicate that complement-mediated virolysis is amplified by the removal of factor H from sera (31). Factor H encourages CFI to cleave activated C3b into iC3b and C3d thereby preventing C3 convertase formation, and thus reducing complement activity during HIV infection (13). Also, the down-regulation of CFI concentration in patients receiving ART enables C3b/C4b attached to gp120/gp41 to intermingle with CR1 on $CD4^{+}$T cells, thereby promoting viral amplification (28).

Our study indicated no significant difference of C4b levels between the HIV-ve and PE-ve pregnancies. The

results are supported by those of Derzsy et al. (32), who also noted similar C4b-binding protein levels amongst PE, normotensive pregnant and non-pregnant women. This is an unexpected finding as C4b should be exaggerated in the pro-inflammatory, pro-apoptotic and anti-angiogenic state of PE. Using a rat model, complement activation and hypertension were associated with induced hypoxia (27). According to Wu et al. (28), in 2014, complement proteins are key protagonists in host immune response to viral infection where C4b promotes HIV replication and amplification. It is also possible that sample type influences C4b expression, as variations of C4b expression has been reported during HIV infection (28). However, the down-regulation of C4b in PE+ve compared to PE-ve pregnancies may be attributed to ART which has been reported to decrease acute phase reactants and regulate complement activation (33).

A dysregulation in C4b has also been linked to an intolerance to sulphonamides and doxycycline (34). Doxycycline has shown to be promising in the treatment of patients with severe acute respiratory syndrome coronavirus-2 infection (34). Consequently, pregnant women with C4b deficiency are at risk if infected with coronavirus, as doxycycline treatment has minimal effectiveness (34). Liesmaa et al. (35), reported a downregulation of C4b in PE linked with adverse outcomes.

Furthermore, C4b plays a significant role in viral infectivity, by enhancing HIV interaction with regulatory CR1 proteins on the surface of $CD4^+$ T cells (36). The CR1 is a co-factor for the inactivation of C4b via CFI, by rapidly dissociating newly formed C4b2a C3 convertase, thus effectively blocking initiation of the complement sequence (12). Deficiency of complement components CI-C4 impaired C3b and C4b formation and led to an accretion of immune complexes in the lymph, extracellular fluid and blood, as well as their retention within tissues (37). Dysregulation of C4b in HIV+ve individuals may arise from deficiencies of upstream precursor complement proteins. Moreover, an increase in herpes simplex viral infection was also found to be associated with C4b deficiency (38).

Remarkably, our findings also demonstrated statistically significant differences for gestational age, maternal weight, parity, maternal age, gravidity, BMI SBP and DBP between the normotensive and PE+ve and PE-ve patients. Considering the associations between demographics and complement analyses C4b and CFI, Mulvihill et al. (39), in 2019, showed that an elevated gene copy number and amplified serum complement levels of C4b were associated with high blood pressure. However, Richani et al. (40), observed no change in these complement components at different gestational ages.

Study Limitations

Shortcomings of our study was a small sample size, term gestational age and the influence of HAART on HIV-positive women. It would be interesting to evaluate C4b and CFI levels throughout gestation with a larger series. Future large multi-centered prospective studies are required to validate our findings. More importantly, single nucleotide polymorphisms of C4b and CFI should be examined in a large cohort study.

Conclusion

This novel study demonstrated a decline of CFI and C4b concentration in the PE vs normotensive pregnancies, regardless of HIV status. Similarly, irrespective of pregnancy type, both CFI and C4b levels were downregulated in both HIV positive and HIV negative groups. Since C4b bioavailability is regulated by CFI, it is plausible to suggest that the dysregulation of CFI increases complement activity enabling C4b to participate in the formation of C3 convertases in uninfected pregnancies. However, in HIV infection, complement mediated virolysis may be attributed to C3 convertase deficiency. Mutations in genes encoding for C4b and CFI may also be responsible for the dysregulation in HIV associated PE pregnancies. Furthermore, in the dyad of HIV associated PE, HAART management may be implicated in both C4b and CFI dysregulation.

Ethics

Ethics Committee Approval: Our study was approved by the Ethics Committee of University of Kwazulu-Natal (approval number: BCA338/17).

Informed Consent: Informed consent from all patients were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.N., Design: T.N., Data Collection or Processing: R.G., T.N., Analysis or Interpretation: R.G., N.G., T.N., Literature Search: R.G., N.G., T.N., Writing: R.G., N.G., T.N.

Conflict of Interest: No conflict of interest was declared by the authors.

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