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Are concentrations of Clusterin and Beta-2-Glycoprotein I dysregulated in HIV Associated Preeclampsia?

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Abstract

Objective

To evaluate the levels of serum beta-2-glycoprotein I (β_2GP1) and clusterin in the duality of Pre-eclampsia and HIV.

Method

Stored serum samples collected from 72 pregnant women were stratified according to the pregnancy type (pre-eclamptic and healthy normotensive groups) and HIV status (positive or negative). A Bio-Plex multiplex immunoassay was used to determine the concentrations of clusterin and β_2GP1.

Results

Clusterin concentrations differed significantly ($p = 0.01$) between the HIV positive (+) (mean= 123 800 ng/ml; 95% CI: 105 400-142 200) *vs.* HIV negative (-) (mean= 92 190 ng /ml; 95%CI: 75 840-108 500) groups and across all groups ($p = 0.0006$). Beta-2-glycoprotein I concentration differed significantly based on HIV status ($p < 0.0001$); HIV+ (mean= 393 649 ng/ml; 95%CI: 30 300-467 000) *vs* HIV- (mean= 224 309 ng/ml; 95%CI: 154 000-294 700) and across all groups ($p < 0.0001$). No significant difference was observed between normotensive and Pre-eclamptic groups for both clusterin and β_2GPI.

Conclusion

Serum concentrations of clusterin and β_2GPI were significantly increased in HIV positive pregnancies. It is postulated that both clusterin and β_2GPI may have a role in HIV disease progression. These findings need to be confirmed in studies having larger sample sizes and detailed information on anti-retroviral therapy.

Keywords

Pre-eclampsia, HIV, Clusterin, β_2GPI

Introduction

Globally, 37.9 million people are HIV infected, hence this is a major health and economic challenge particularly in low- and middle -income countries (LMIC) (1). HIV infection and it's co-morbid diseases are also the leading cause of maternal deaths in the world with approximately 17.4 million women being infected (2). In South Africa (SA), 13.5% (7.97 million) of the overall population are HIV infected (3). Additionally, approximately 20% of the female population in their reproductive age are HIV infected (3).

Pre-eclampsia (PE) co-morbid with HIV infection has a high prevalence in LMIC (4,5). In SA, HIV infection and PE are the major direct causes of maternal and neonatal morbidity and mortality mainly due to late booking, sub-optimal care and poor access to health care facilities (6). The prevalence of PE may be affected by HIV infection and by antiretroviral therapy (7, 8). The use of highly active antiretroviral therapy during pregnancy reconstitutes the immune response exacerbating the pro-inflammatory micro-environment of PE (9). Pre-eclampsia is a pregnancy-specific disorder characterized by the new-onset hypertension (Blood Pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic) at or after 20 weeks gestation combined with the development of one or more of the following conditions: proteinuria, haemolysis, neurological features, liver dysfunction, fetal growth restriction, thrombocytopenia or acute kidney injury (10). However, the pathogenesis and etiology of PE remains unclear (7,11,12). Immune and vascular dysregulation together with endothelial pathology are recognized factors which underlie the defective placentation and exaggerated inflammation of the placenta associated with PE (5,12).

Pregnancy is associated with fat accumulation during the early weeks of gestation, lipid metabolism and degradation occur to sustain the nutrient demands of the fetus (13). Changes in the maternal lipid profile include a progressive increase in the circulating levels of total cholesterol (TChol), LDL cholesterol (LDLc) and triglycerides during gestation including the concentration of HDL-cholesterol (HDLc) which also increases until the middle of pregnancy, and thereafter slightly decreases (14,15). Dyslipidemia is related to polymorphisms of apolipoprotein that contribute to endothelial cell dysfunction (16). Hence apolipoproteins are plausible contributors to the endothelial dysfunction that characterises PE development (17). In PE, the hyperlipidemia observed as the ApoB/ApoA-I ratio contributes to endothelial dysfunction via oxidative stress (18).

Apolipoprotein J also called clusterin (CLU) is a multifunctional glycoprotein that is involved in many physiological and pathological states, such as cancer and Alzheimer's disease (19). It is a 70 to 80-kDa disulphide-linked heterodimeric glycoprotein commonly found in animal tissues and body fluids (20). Clusterin consists of two chains, α- and β-clusterin (21) . There are two isoforms: a precursor nuclear and a secreted extracellular form (21). During oxidative stress and endothelial injury, the nuclear form is associated with cell death and apoptosis whereas the secreted form has a protective response (14). Cheng *et al*., (2015) reported an up-regulation of clusterin in PE development emanating from its biological characteristics (22). Similarly, Watanabe *et al.*, (2004) reported elevated serum clusterin in PE. Despite these investigations, the role of clusterin in the etiology and/or pathogenesis of PE remains unknown particularly when PE is comorbid with HIV infection.

Apolipoprotein H also referred to as beta-2-glycoprotein I (β_2GPI), is a plasma glycoprotein occurring as a free protein or associated with other lipoproteins (23–25). Beta-2-glycoprotein I is a 54-kDa single-chain glycoprotein with 5 carbohydrate chains and 326 amino acid residues (23). The protein consists of five internal repeat domains of 60 amino acid residues, each with internal disulphide bonds known as the Sushi domain (26,27). Beta-2-glycoprotein I is a complement regulatory protein with an anti-coagulation activity that occurs by inhibition of factor XI/XIa and thrombin (28). Furthermore, it inhibits protein C and serotonin release and has anti-angiogenic activity (29–31). Additionally, β_2GP1is the primary antigen to bind anti-phospholipid hence is often associated with antiphospholipid syndrome (APS), venous and arterial thrombosis, fetal loss and PE (28). *In vivo,* β_2GP1binds to phosphatidylserine on the trophoblast cell membrane, platelets, and endothelial cells hence its biological function in the defective trophoblast invasion that characterises PE (24,27,32).

In light of the paucity of information of clusterin and β_2GPI in PE co-morbid with HIV infection, this study aims to evaluate the levels of serum β_2GPI and clusterin in the duality of PE and HIV infection.

Materials and Methods

Ethical considerations

This study obtained institutional ethics approval and was appended to the primary study (BCA 338/17) for use stored samples.

Study Population

The study population (n=72) consisted of normotensive pregnant (n=36) and pre-eclamptic (n= 36) women. Each group was further sub-stratified by HIV status into HIV positive and HIV negative (n=18 each). Sample size was determined using Cohen's formula for effect size. The Bioplex assay was performed in duplicate

Inclusion Criteria

Pre-eclampsia was defined as the presence of newly-onset blood pressure of ≥140 mmHg systolic and/or ≥90 mmHg diastolic and proteinuria (+1 urine dipstick test) (10). Early-onset pre-eclampsia (EOPE) was diagnosed on appearance of clinical signs and symptoms before 34 weeks gestation (22). A rapid bed-side finger prick test was used to determine HIV status. HIV positive women on antiretroviral therapy were included.

Exclusion criteria

Participants with chronic hypertension, abruption placentae, pregestational/gestational diabetes, systemic lupus erythematosus, sickle cell disease eclampsia, thyroid disease intrauterine death, chronic renal disease, anti-phospholipid antibody syndrome and cardiac disease. Participants who were treated with aspirin, asthma medication and non-steroidal anti-inflammatory drugs were also excluded.

Methods

Maternal whole blood samples were collected and centrifuged (3000 rpm for 10 mins at 4°C). Upon separation, the supernatant was collected and stored at -80°C. For the purpose of this study, serum samples were utilised for the quantification of clusterin and β_2GPI.

BioPlex Multiplex Assay

A Bio-Plex Pro Human Apolipoprotein 10-plex assay was used according to the manufacturer's instructions (Bio-Rad Laboratories, Inc., USA).

The serum samples were centrifuged (1000 g for 15 mins at 4°C) and thereafter a 1:50 000 dilution was prepared using a sample dilution buffer. The standards, controls (1 and 2), blocking buffer, standard diluent and detection antibodies were reconstituted with distilled water. These reconstituted products were then left to rest for 5 mins and subsequently vortexed. The standards were prepared in a 1:3 serial dilution.

A blocking agent was added to the provided 96 well plate, followed by standards, controls, samples and blanks. Thereafter, capture beads were added to the plate. Post-incubation on a shaker (1hr) reconstituted detection antibodies were added. Streptavidin-phycoerythrin (SA-PE) was added to complete the reaction. The final incubation (30 mins) was performed with subsequent plate washing (3x) followed by resuspending the beads with assay buffer. Post incubation (30s), the plate was read on the Bio-Plex "MAGPIX" Multiple Reader and data was obtained using the Bio-Plex Manager software version 4.1 (Bio-Rad Laboratories, Inc., USA).

Statistical Analysis

GraphPad Prism v5.00 for Windows (GraphPad Software, San Diego California USA) was used to analyse data. The Kolmogorov Smirnov normality test was performed, and data were found to be non-parametrically distributed. Statistical significance was determined using a Mann-Whitney U test or Kruskal-Wallis test. For multiple group comparisons an ANOVA, followed by the Bonferroni post-hoc test was performed. A probability level of $p < 0.05$ was considered statistically significant.

Results

Clinical Characteristics

Patient demographics of the study population at term are shown in Table 1. Gestational age [($p < 0.0001$)(PE+:31-37, PE-:33-38, N+:38-40, N-:39=40)] systolic [($p < 0.0001$) (PE+:156-177, PE-:161-177, N+:116-127, N-:107-124)] and diastolic [($p < 0.0001$) (PE+:97-113, PE-:98-111, N+:62-85, N-:63-74)] blood pressures and maternal weight [($p = 0.03$) (PE+:67-80,PE-:68-99, N+:65-73,N-:66-85)] were statistically different between the N versus PE group.

Serum concentration of Clusterin

Pregnancy type:

When comparing clusterin levels in PE *vs.* N groups irrespective of HIV status, no significant difference was noted [(p = 0.51); PE (mean= 107 300 ng/ml) *vs* N (mean= 108 700 ng/ml) Table 2 and Figure 1A].

HIV status:

Serum concentration of clusterin significantly differed between HIV+ and HIV- patients regardless of pregnancy type [(p = 0.01); HIV+ (mean=123 800 ng/ml) *vs* HIV- (mean= 92 190 ng/ml) Figure 1B].

Across all groups:

Clusterin was statistically different across the study groups (p = 0.0006). Specifically, a significant difference was reported between PE+ (mean = 139 746ng/ml) vs PE- (mean = 109 583 ng/ml) [p = 0.0002]; *vs*; and PE- (mean = 107 859 ng/ml) *vs* N- (mean = 74 792 ng/ml) groups [p = 0.006] (Table 2; Figure 1C).

Serum concentration of β2GPI

Pregnancy type:

Regardless of HIV status, no significant difference (p = 0.91) of β_2GPI concentration was noted in PE (mean= 308 900 ng/ml) *vs* N (mean= 309 100 ng/ml) groups (Table 3 and Figure 2A).

HIV status:

Irrespective of pregnancy type, a significant difference of β_2GPI concentration was reported between the HIV+ and HIV- groups [(p < 0.0001); (mean= 393 649 ng/ml) *vs* (mean= 224 309 ng/ml); Figure 2B)].

Across all groups:

A statistically significant difference of β_2GPI concentration was noted across all groups (p < 0.0001). A significant difference (p < 0.0001) of β2GPI concentration was demonstrated between PE+ (mean = 144 819 ng/ml) *vs* PE- (mean = 303 798 ng/ml); PE+ (mean = 303 798ng/ml) *vs* N- (mean = 314 327 ng/ml) [(p = 0.002); and PE- (mean = 314 327 ng/ml) *vs* N+ (mean = 472 971 ng/ml) groups [(p = 0.0001); Table 3 and Figure 2C].

Discussion

In our study, based on pregnancy type, clusterin concentration was not statistically different between PE and N groups (p = 0.51), irrespective of HIV status albeit with a decreased in PE compared. Clusterin is implicated in pathogenic conditions mostly related to oxidative stress (33). This unexpected result within a hypoxic placental micro-environment may be attributed to the duality of HIV infection co-morbid with PE. Nonetheless, inositol requiring enzyme-1 knockdown of glioma cells down-regulate the expression of clusterin gene and regulates their hypoxic response (34).

Clusterin gene expression is also regulated by NF-κB, growth factors and cytokines; and stress- or apoptotic-inducing agents (35). Notably, clusterin has antioxidant properties and may protect cells from apoptosis induced by reactive oxygen species (15,36). It has been suggested that secretory clusterin acts as an extracellular molecular chaperone, scavenging misfolded or denatured proteins that are produced following stress-induced injury (36–38).

Pre-eclampsia represents an exaggerated inflammatory response and is associated with excessive complement activation, particularly the terminal complement complex (C5b-9) and C5a (39,40). The downregulated trend of clusterin in PE in our study may be attributed to its' role as a complement inhibitor (41). Moreover, the hyper-inflammatory micro-environment of PE may activate the inhibition of the membrane attack complex of complement proteins (41). It has also been suggested that the binding of clusterin to hydrophobic regions on unfolded, stressed proteins would prevent aggregation (42).

In contrast to our findings, Shin *et al.,* (2008) reported an increased clusterin expression in PE compared to normal pregnancy (20). A similar elevation of serum clusterin levels in PE was previously attributed an aberrant regulation of clusterin gene (43). A significant difference in clusterin between PE and normotensive groups were observed by Odun-Ayo *et al.,* (2018) albeit in urinary samples (33).

Pre-eclampsia has been recognized to have an hypoxic micro-environment (44). Clusterin may have a cytoprotective function against the damaging effects of hypoxia and oxidants such as H_2O_2, superoxide anions, and oxidative stress-induced apoptosis acting via the Akt/GSK-3β signalling pathways (45).

Based on HIV status, regardless of pregnancy type (PE or N), we report a significant upregulation of clusterin between HIV+ compared to HIV- groups (p = 0.0094). This

dysregulation of clusterin protein expression may be induced by HIV accessory protein such as Env, Tat and Rev7 and the LTR (43). Our results are in contrast with Odun-Ayo et al., (2018) who examined urinary clusterin (33). Clusterin is a stress-induced protein can be triggered by a variety of factors such as viral infection and oxygen radicals (33). Clusterin is a protease inhibitor that is important in viral transmission acting to prevent viral infection and replication (46).

Based on pregnancy type, β_2GPI concentration was not statistically different between PE compared to N pregnant women (p = 0.91) irrespective of HIV status: however, there was a downward trend in the concentration of β_2GPI. These results are similar to that of Watanabe et al., (2004) who reported no significant difference between severe preeclamptic compared to normotensive pregnant age-matched and gestational aged matched women (47). Taking into account the heterogeneity of PE , the strength of our study was the inclusion of EOPE, the sub type of PE associated with high rates of maternal and perinatal morbidity and mortality (10).

Systemic lupus erythromatosis, a similar hyperinflammatory state to PE is characterized by high serum β_2GPI complexes designating significant vascular oxidative stress (48). Notably, our results are unexpected, as PE is associated with inflammation and vascular dysregulation that occurs within the hypoxic, oxidative stressed micro-environment (48).

Pre-eclampsia is associated with widespread endotheliosis lesion (49). β_2GPI is a thiol oxidoreductase substrate that protects endothelial cells from oxidative stress-induced cell death (30). Under oxidative stress conditions such as PE, free thiol β_2GPI undergoes posttranslational change via oxidation and/or nitrolysation of its cysteine residues (49). It is possible that the lack of a significant difference based on pregnancy type may be attributed to the duality/synergy of HIV infection and PE.

Importantly, it is widely accepted that PE is an anti-angiogenic state (50). Cumulative evidence suggests that maternal angiogenic shift occurs in favour of elevated production of sFlt-1 and soluble endoglin (50). The decrease of β2GPI in PE in our study may be a compensatory response to the hypoxic oxidatively stressed and antiangiogenic micro-environment. Importantly all HIV patients in our study received anti-retroviral therapy which may have neutralised the immune and angiogenic response.

Based on HIV status there was a significant up-regulation of β_2GPI in HIV+ and HIV–, regardless of pregnancy type (Figure 2B). It has been previously reported that β_2GPI binds to retroviral HIV antigens, p18 Gag protein 1, p26 Gag protein, and to gp160 of HIV-1 (51). Other plasma proteins, including apolipoprotein AI, have been shown to bind HIV-1 viral protein (51). This binding capacity demonstrates that β_2GPI is a part of our innate immune response (51). The latter binding is depend on both pH and ionic strength, with optimum pH 5.6 and 50Mm NaCl (51). However, western blot results show poor interaction of β_2GPI to gp120 and gp160, yet strong interactions were observed by ELISA (51).

In our study, we noted a statistical difference of β_2GPI across study groups, albeit with an elevation in the concentration of β_2GPI in PE+ *vs* PE-, PE+ *vs* N- and PE-*vs* N+. β_2GPI has five domains that are linked to a phospholipid component of the cell membrane (26). It has been reported that anti-phospholipid antibodies play a role in HIV-related immunopathogenesis (52). In the setting of HIV infection, an increased rate of spontaneous apoptosis in peripheral blood lymphocytes was reported (53). In HIV infected patients lymphocyte apoptosis is associated with alterations of the mitochondrial function such as changes of mitochondrial transmembrane potential and increased mitochondrial generation of superoxide anions (52,53). In addition, a structural defect of the cardiolipin-containing inner mitochondrial membrane of lymphocytes occurs in HIV-infected individuals(52,53). Structurally altered mitochondrial phospholipids are transported by a phospholipid flippase/translocase mechanism to the cell surface and then act as new antigens which induce a humoral immune response in the HIV-infected host who already present with polyclonal B cell (52,54,55).

The relationship between PE and HIV infection requires demystifying due to opposing immune response (56). Studies have reported a protective effect of HIV infection in PE development (7,9). Highly active anti-retroviral treatment (HAART) is the standard treatment for HIV positive pregnant and non-pregnant women in South Africa (56). Before the arrival of HAART, PE was uncommon in HIV positive pregnant women, being lower than in the general population (57). However, the usage of HAART may increase the risk of development of PE (57).

In our study, gestational age was statistically different between N vs PE. Notably, the effect of PE on birth weight is a function of gestational age (58). In contrast, maternal weight was similar between study groups. Our results are unexpected as previous studies have shown that maternal weight is a risk factor for PE development (59). Limitations of this study may include the small

sample size, absence of viral load data as well as the duration of HAART (pre- or intra-pregnancy).

In conclusion, this study demonstrates that based on HIV status, an elevation of β_2GPI and clusterin occurs in HIV infected pregnant women. It is plausible these stress-induced glycoproteins are activated by viral load and interaction with HIV accessory proteins. Surprisingly, β_2GPI and clusterin levels were similar between PE and normotensive pregnancies, it is therefore plausible that the synergy of HIV infection comorbid with PE and/or antiretroviral therapy may impact the glycoprotein levels. We recommend a larger sample size for the consideration of clusterin and β_2GPI in the prediction of PE development.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

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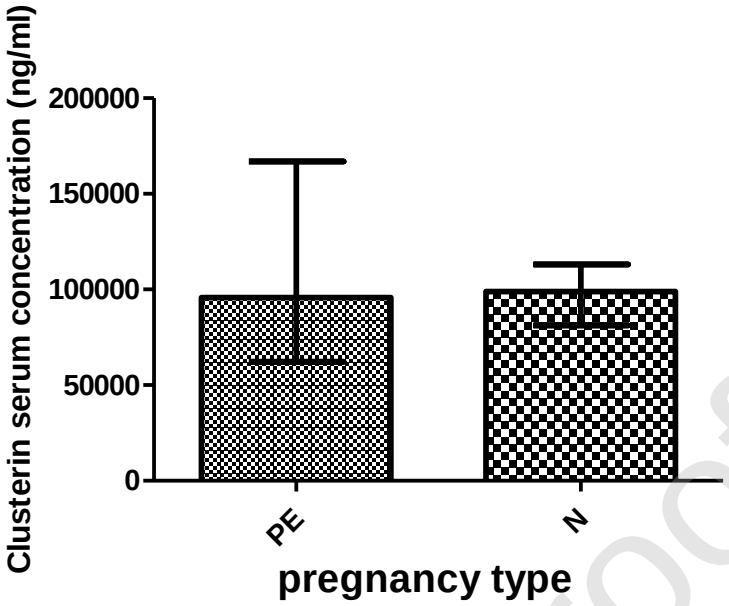

Figure 1A. Serum concentration of Clusterin (ng/ml) pregnancy type

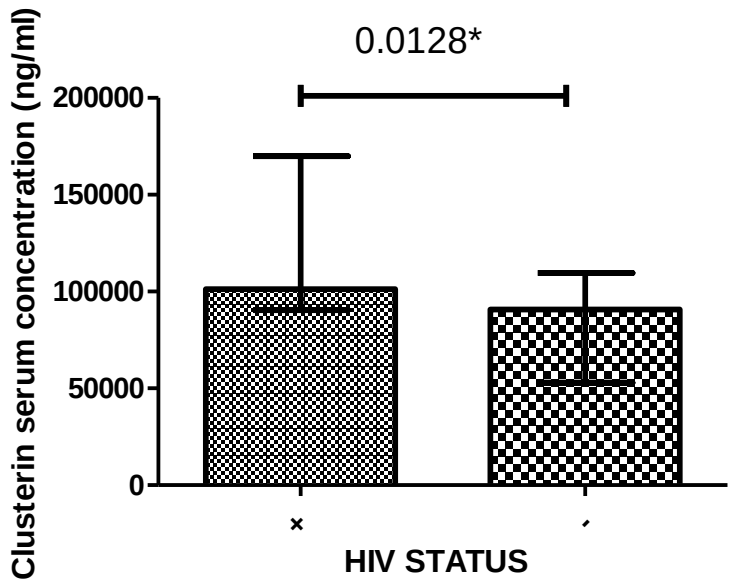

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Figure 1B. Serum concentration of Clusterin (ng/ml) reported in HIV status.

* $p < 0.05$

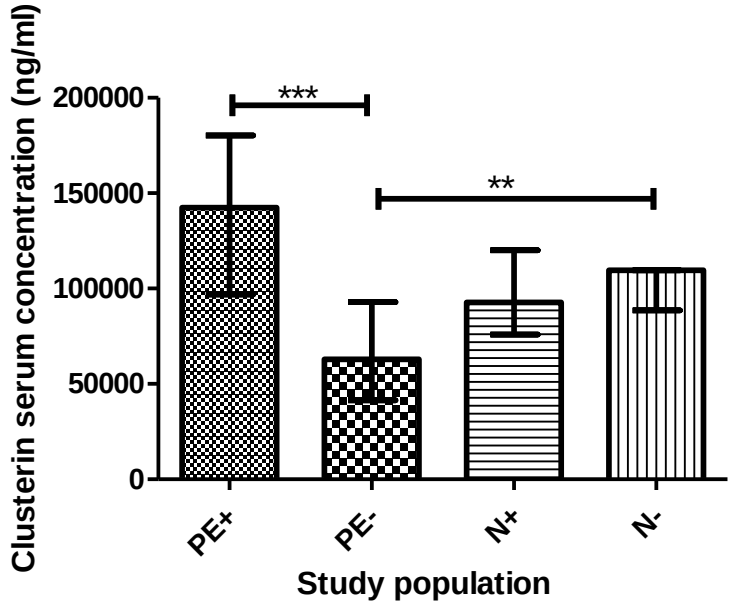

Figure 1C. Serum concentration of Clusterin (ng/ml) reported in the study population.

** $p < 0.01$

*** $p < 0.001$

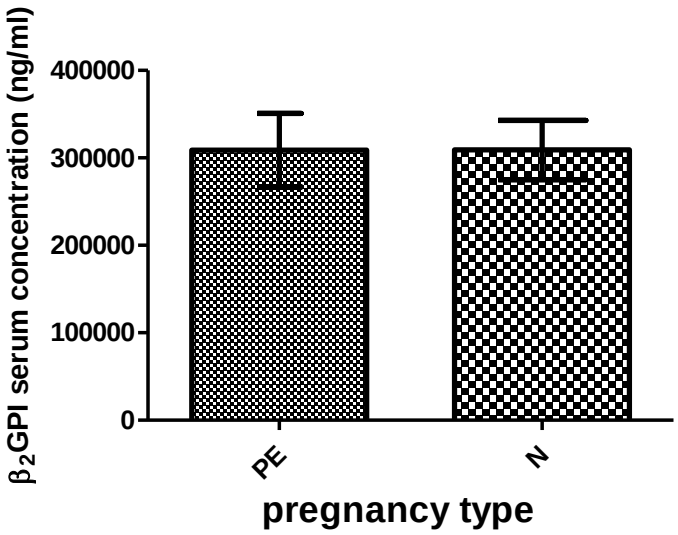

Figure 2A. Serum concentration of β_2GPI (ng/ml) pregnancy type

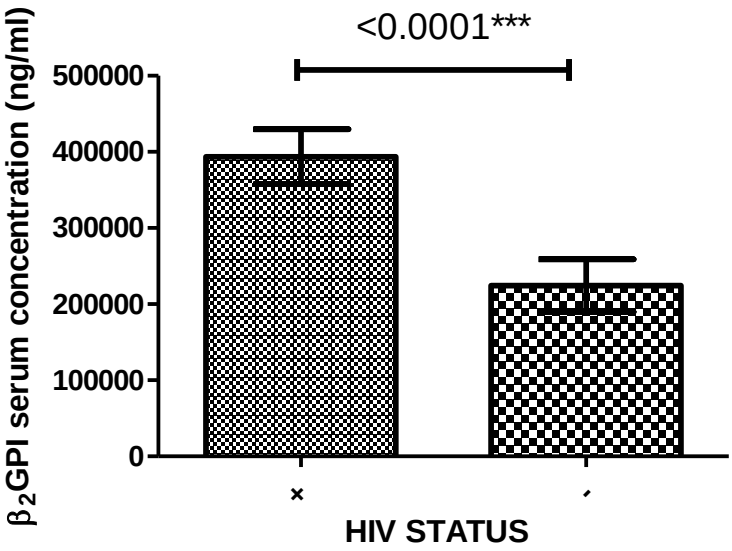

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Figure 2B. Serum concentration of β_2GPI (ng/ml) reported in HIV status.

*** $p < 0.001$

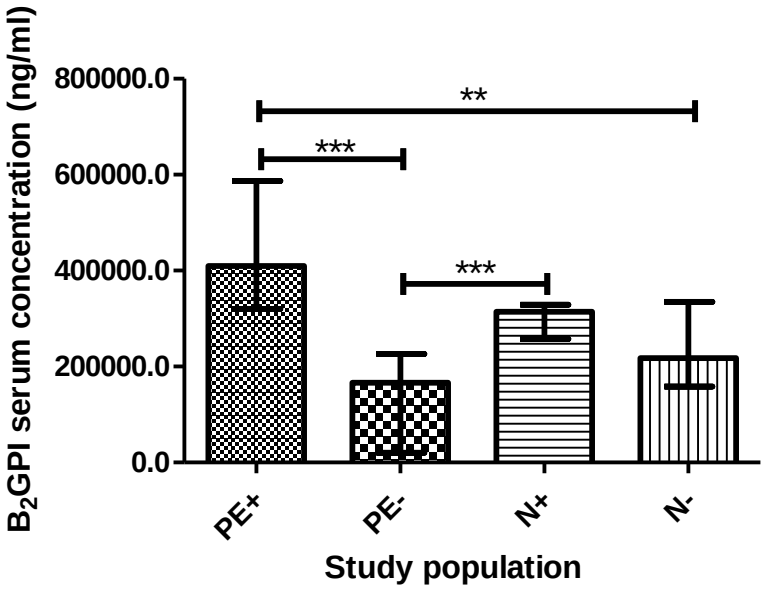

Figure 2C: Serum concentration of B_2GPI (ng/ml) reported in the study population

** $p < 0.01$

*** $p < 0.001$

Table 1. Clinical characteristics of the study population

		Median	**Q3-Q1**	**Mean±SD**	***p*** **all groups**	***p*** **PE *vs* N**
Systolic BP (mmHg)	PE+	167.5	156-177.3	166.8±12.3	< 0.0001	< 0.0001
	PE-	168	160.5-176.5	170.6±14.7		
	N+	120.5	116-127.3	120.1±9.5		
	N-	119	107-124.8	115.2±10.9		
Diastolic BP (mmHg)	PE+	103.5	97-113.3	106±14.2	< 0.0001	< 0.0001
	PE-	105.5	98-111.3	104.6±13.6		
	N+	76.5	62-85.3	72±15.3		
	N-	70	63.8-74.5	68.1±8.6		
Maternal weight (kg)	PE+	69.5	67.4-80.6	76.3±15	0.05	0.03
	PE-	75.5	68.3-98.5	81.9±21		
	N+	68	65.05-72.7	68.2±7		
	N-	75	85-67	75.1±10.8		
Maternal age (years)	PE+	29	30-26.3	27.8±4.6	0.85	0.38
	PE-	25	21-32	27.2±7.5		
	N+	27.5	24.5-34	29.3±6.6		
	N-	24.5	19.3-36.5	29.3±12.7		
Parity	PE+	1	1-2	1.3±0.9	0.61	0.53
	PE-	1	0-2	1.2±1.3		
	N+	2	1-3	1.7±1.4		
	N-	1	0-2	1.2±1.5		
Gravidity	PE+	2	1.3-3	2.2±1.1	0.01	0.64
	PE-	2	1-3	2.1±1.1		
	N+	3	1-3	2.5±1.3		
	N-	1	1-1	1.1±0.3		
Gestational age (weeks)	PE+	32	37-31	32.9±4.5	< 0.0001	< 0.0001
	PE-	35	33-38	34.5±4.4		
	N+	39	38-40	38.8±1.4		
	N-	40	39-40	39.4±1.3		

Mean±SD: Mean±standard deviation; PE+: pre-eclamptic HIV positive; PE-: pre-eclamptic HIV negative; N+: normotensive HIV positive; N-: normotensive HIV negative

Table 2. Observed concentrations of Clusterin (ng/ml) in the study population

	PE+	**PE-**	**N+**	**N-**
n	18	18	18	18
Mean±SD	139 746±45 013	74 792±47 595	107 859±59 624	109 583±43 557
Median	142 399	62 939	92 819	109 583
Q3-Q1	180 207-96 927	92 863-41 472	120 100-75 946	109 583-88 589
***p* all groups**	0.0006			
***p* PE *vs* N**	0.5084			

Mean±SD: Mean±standard deviation; PE+: pre-eclamptic HIV positive; PE-: pre-eclamptic HIV negative;
N+: normotensive HIV positive; N-: normotensive HIV negative

Table 3. Observed concentrations of B_2GPI (ng/ml) in the study population

	PE+	**PE-**	**N+**	**N-**
N	18	18	18	18
Mean±SD	47 2971±251 223	144 819±103 831	314 327±142 245	303 798±254 681
Median	409 626	166 320	314 327	217 658
Q3-Q1	586 686-320 455	226 087-20 100	328 717-257 976	334 742-158 473
***p* all groups**	< 0.0001			
***p* PE *vs* N**	0.9059			

Mean±SD: Mean±standard deviation; PE+: pre-eclamptic HIV positive; PE-: pre-eclamptic HIV negative;
N+: normotensive HIV positive; N-: normotensive HIV negative