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RELATIONSHIP OF BLOOD PRESSURE AND HEMORHEOLOGICAL FACTORS TO GESTATIONAL AGE IN NORMAL HUMAN PREGNANCY

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ABSTRACT

Normal human pregnancy (PREG) predisposes towards gestational age (GA) based manifestation of atherogenic cardiovascular disease (CAD) risk [3]. This study thus relates the dynamics of blood pressure (BP) and hemorheological (HH) factors, known CAD risk factors to GA in 50 normal pregnant human females at GA less than 20wk (PREG < 20 wk) and GA 20wk and above (PREG ≥ 20wk). 25 apparently healthy non-pregnant (NO PREG) subjects served as Control. Systolic BP (SBP) and diastolic BP (DBP) were determined in sitting position with a mercury sphygmomanometer. Pulse pressure (PP) was calculated as SBP less DBP. Mean arterial pressure (MAP) was estimated as summed DPB and one third PP. Total plasma protein (TPP) concentration was determined enzynmatically. Levels of hematological variables were determined electronically. Relative plasma viscosity (RPv) and red blood cell (RBC) viscosity (RBv) were measured by simple needle and syringe type viscometry[9]. RBC deformability index (DI) was computed as quotient of corrected RBv and RPv[10]. Significant drop in SBP was noted early in PREG group compared with the control ($p<0.05$). At PREG ≥ 20wk, SBP increased towards control value. Similar trend was observed in DBP pattern. MAP was significantly ($p<0.05$) depressed in PREG commencing early in PREG (PREG < 20 wk). No significant differences were noticed in hemoglobin concentrations of PREG and Control. Compared to Control, significant decreases were observed in values of hematocrit, platelet count and TPP concentrations of PREG groups ($p<0.05$). White blood cell (Wbc) count was significantly ($p<0.05$) elevated in PREG group compared to Control. PREG ≥ 20wk Wbc was significantly higher than PREG < 20 wk ($p<0.05$). PREG ≥ 20wk RPv was significantly higher than Control or PREG < 20 wk ($p<0.05$). Compared to Control, PREG ≥ 20wk RBv was significantly ($p<0.05$) lower than PREG < 20 wk. Percent DI was similarly significantly ($P<0.05$) lower in PREG ≥ 20wk vis-a-vis PREG < 20 wk or Control. These results potently suggest that the HH changes observed in this study are of advantage to the hyper-dynamic circulation of PREG. These changes in conjunction with falls in BP prevent the potential progression of normal PREG-induced CAD risk onto frank CAD.

INTRODUCTION

Fundamental to the current global stride towards reducing (or eliminating) maternal mortality during child birth are thorough understanding of the full dynamics of normal pregnancy (PREG) and its associated morbidity, as well as attaining safe and uncomplicated PREG. Normal human PREG is a transient hyper-physiologic state. Large- scale changes of physiological functions affecting all body systems are induced in preparation for and adaptation to accommodate and support

KEY WORDS: *Blood-pressure; Hemorheological- factors; Gestational -age; Normal- human- pregnancy*

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the developing fetus[1]. Due to the massive challenge especially to the endocrine system, early in PREG there is a massive increase in body fat accumulation and equally rapid lipid and lipoproteins turnover[2]. In concert with other factors including mechanical ones, the PREG/ endocrine challenge also induces other cardiovascular and blood changes with potential cardiovascular disease (CAD) outcome.

Results from a recent study[3]have demonstrated that normal PREG potentially predisposes towards atherogenic CAD risk as the PREG progressed. Alterations in maternal hemodynamics occur early in normal PREG and are maintained into late PREG[4]. Because of the elevated hormonal stance, PREG generally is a hypervolemic state. It is accompanied by early- expanded volume, an augmented maternal cardiac output (CO) and a diminished vascular and rheological resistance[5]. These normal PREG - induced changes are transient and produce no detrimental effect on the mother. At term, factors yet to be fully delineated prevent the progression of these PREG induced CAD risk onto frank CAD[3]. These types of studies raise basic questions as to the factors which moderate the manifestation of CAD risk in normal PREG. A host of hematological studies have evaluated blood rheological flow including erythrocytic deformability in health or in sickness. Such studies established these variables as known indices of CAD[6]. For example, poor blood viscosity, red blood cell (RBC) aggregation and poor rheology either separately or collectively are associated with CAD risk generally[7]. Furthermore, a limited number of studies on physiological changes in hemorheology of normal PREG have demonstrated rises in fibrinogen level, increases in RBC aggregation and diminished RBC deformability[5,8]. These potential CAD risk are however balanced by the hemodilution with a concomitant reduction in hematocrit, consequent upon standing hypervolemia of the PREG. Finally, in addition to being sparse, the majority of these available studies on cardiorheological risk, measured these risk variables usually in the second and third trimesters of PREG. Thus the data are less reflective of events spanning the entire spectrum of normal PREG. Therefore, there still exists paucity of correlative data on hemorheological (HH) CAD risk factor especially commencing from the onset of PREG. An evaluation of such potential CAD risk factor in normal PREG may thus pave the way for a more comprehensive understanding of the dynamics of PREG induced CAD risk and morbidity or otherwise.

This study therefore sought especially to relate blood pressure, and HH factors, known CAD risk factors to gestational age (GA) in normal human PREG.

SUBJECTS

Subjects and Selection criteria: Fifty healthy pregnant women of informed consent, attending the Ante-natal clinic at the maternity wing of the University of Ilorin Teaching Hospital of ages between 19-40 years participated in the study. Twenty-five (25) subjects each of the two study groups who were of PREG at GA of less than 20 weeks (PREG < 20 wk) and 20 weeks or more (PREG ≥ 20 wk) were randomly selected. 25 apparently healthy non-pregnant (No PREG) subjects, matched for age, parity and body mass index (BMI), mostly staff of the University of Ilorin served as the Control.

None of the subjects from the Records and Assessment of the Ante-natal Clinic had any cardiovascular, inflammatory or renal disease. The subjects had neither an established cholestasis, diabetes mellitus or gestational diabetes nor any known obstetric complications such as pre-eclampsia, eclampsia or polyhydraminions. They were not on (and were counseled against) any medications including oral and ingestible contraceptives in course of the study. Smokers and alcoholics or subjects who were not sure of their last menstrual period were also excluded from the study. All studies were conducted in the morning hours from 9:00AM after overnight fast. All the subjects gave their informed consent to participate in the study. The study was performed in accordance with the Helsinki declaration and international norms, and also with the standards of the local University Research and Ethics Committee.

METHODS

Anthropometric measurement and Obstetric History:

Anthropometric measurements as well as relevant obstetric history such as parity were determined. The subjects' bodyweight (Bwt) and height were recorded without shoes. Body mass index (BMI, kg/m^2) was calculated. These are summarized in Table 1.

Determination of blood pressure

The measurement of blood pressure (BP) began following a twenty minute rest period. BP was measured in sitting position. Systolic BP (SBP) and diastolic BP (DBP) were determined using a standard mercury sphygmomanometer. The first and fifth Krokotoff phases were taken to represent the SBP and DBP respectively. Pulse pressure (PP) was determined by subtracting the DBP from the SBP. Mean arterial blood pressure (MAP) was then computed as summed one third PP and DBP.

Blood collection and preparation of plasma samples:

Following overnight fast, a total of 3ml of venous blood samples were asceptically collected through a clean venopuncture by clinically qualified staff of the Ante-natal Clinic for the determination of total plasma protein concentration and hematological (HT) parameters. The samples were collected into 5ml EDTA vacutainers. Collected blood samples were subsequently spun at 3000rpm for 10minutes to yield clean hemolysis free plasma. The plasma samples were separated and stored frozen in aliquots until analyzed usually in triplicates.

Measurement of total plasma protein concentration:

Total plasma protein (TPP) concentration was established by micro biuret method using Randox protein determination kit, TP245 by Randox laboratory, Antrim UK., following the manufacturer's instructions. Assay results were read spectrophotometrically at an optical density of 546nm.

Determination of hematological parameters

Levels of HT variables of the study subjects were determined electronically employing the automated HT analyzer, SYSMEX KX-21 (SYSMEX CORPORATION, JAPAN) available in the hematology Department of the University of Ilorin Teaching Hospital, Ilorin. Measured HT variables included hematocrit (Hct, %), hemoglobin (Hb) concentration (g/dl), white blood cell (Wbc) count (x 10^9/L) and platelet count (x 10^9/L).

Determination of plasma or whole blood viscosity:

Plasma and whole blood viscosities were measured using the simple needle and syringe viscometry[9] maintained at 37°C in a thermostatically controlled incubator (Bisca model: cella termostatia Ad-Aqua, BE-89).

Plasma or whole blood viscosity is expressed as relative plasma or whole blood viscosity, and it is the ratio of the flow time (t) of plasma or whole blood (P_t or B_t) to the flow time of an equi-volume of distilled water (W_t). Mean flow time was derived following 20 successive runs.

Relative viscosity (R_v) was obtained from the formula:

$$RP_v = P_t \div W_t \quad \text{or} \quad RB_v = B_t \div W_t$$

where: P_t, B_t and W_t are flow time in seconds for plasma, blood and distilled water respectively.

Red Blood Cell Deformability Index:

RBC Deformability index (DI) was computed as the quotient of corrected RB_v and RP_v.[10]

Data Handling and Statistical Analysis:

All values of variables are reported as mean ± standard error of the mean (SEM). Statistical comparison was by analysis of variance (ANOVA) with graphical post hoc test of significance. A $p < 0.05$ was considered statistically significant [11, 12, 13].

RESULTS

The anthropometrical and obstetric measurements of the study subjects are summarized in Table 1. The table validates the choice of the study subjects. There were no statistically significant variance in the study subjects as to linear age, height and parity. Similarly, no statistically significant differences were noted in the bodyweight (Bwt) and body mass index (BMI) of the pregnant groups.

Blood pressure profile of the study subjects is summarized in Table 2. An initial statistically significant drop in SBP was noted in the PREG groups when compared with the No PREG group ($p<0.05$). Later in PREG (PREG≥20wk), SBP increased towards the control value. Similar trend was observed in the DBP pattern. Overall as depicted in Table 2, the MAP was significantly ($p<0.05$) depressed in PREG commencing especially from the early PREG (PREG < 20wk) in comparison with Control.

Table 3 depicts HT parameters including the TPP concentrations of the studied groups. No statistically significant differences were noticed in the Hb concentrations of the pregnant and non-pregnant subjects. Compared to control, significant decreases ($p<0.05$) were observed in the values of Hct and platelet count of the pregnant groups starting from early PREG (PREG < 20wk). The value of the latter variable was almost half that of the Control in late PREG (PREG≥20wk) A significant ($p<0.05$) decrease was noticed in the TPP concentrations of the pregnant subjects starting early in PREG compared with the Controls. The Wbc count was significantly ($p<0.05$) elevated in the pregnant subjects compared to the Controls. In addition, compared with PREG < 20wk, Wbc values were significantly higher in the PREG (PREG≥20wk) group (Table 3).

The relative plasma and blood viscosity as well as RBC DI of the study subjects are displayed in Table 4. These HH indices of the study subjects exhibited a GA bias. Compared to Control, statistically

significant ($p<0.05$) increases were observed in the pregnant subjects from the onset of PREG. The RPv values of the PREG≥20wk group were significantly higher than those of Control or PREG < 20wk ($p<0.05$). In contrast, RBv exhibited a trend opposite to that of RP_v. Compared to Control, RBv significantly ($p<0.05$) diminished with increasing GA. The PREG≥20wk RB_v value were significantly lower than those of PREG < 20wk or the Control groups ($p<0.05$). The percent DI was similarly significantly ($p<0.05$) lower in PREG≥20wk vis-à-vis PREG ≥20wk or the Control (Table 4)

DISCUSSION

Recent experimental data suggest that normal human pregnancy predisposes towards GA based manifestation of cardiovascular disease (CAD) risk factor. Further, that at term factors endogenous to a normal pregnant human female prevent the progression of these PREG induced CAD risk onto frank CAD[3]. Thus recent efforts have focused on understanding the dynamics of some of these factors in course of normal PREG. This current study relates the dynamics of blood pressure and HH factors, known CAD risk factors to GA of normal pregnant human female.

The results of this study has demonstrated a phasic shift in BP pattern in course of normal PREG. Both the SBP and DBP in this study showed an initial decrease in the pregnant state. This was followed by a subsequent rise towards the control value in the latter part of PREG ≥20wk MAP was similarly depressed commencing early in PREG (PREG < 20wk).

These findings are consistent with published data. A decline in DBP has been demonstrated in early normal PREG and falls to its lowest value at 16-20wk of gestation[14]. These reductions in pressures including SBP become most prominent in mid gestation and then rise gradually to normal levels near term[1]. Mechanistically, the diminished BP in the face of standing hypervolemia of PREG together with an increased CO suggests a diminished peripheral arterial resistance (PAR). This is possibly brought about by a host of factors. Firstly, the diminished PAR is probably reflective of the massive endocrine challenge of the pregnant state[3]; for example a progesterone effect. Increased circulating progesterone acting perhaps through the stimulation of vasodilatory prostaglandin production will help in the diminution of PAR and the MAP[15]. Furthermore, the manifest endocrine challenge would also augment the observed fall in MAP. The later possibly accomplished through the effect of various other pressure agents, circulating Angiotensin II for instance. Secondly, evidence from animal PREG suggests increases in nitric oxide synthase activity of vascular tissues[16,17]. In human PREG, lack of control for dietary variation makes similar observation more difficult. However, studies have suggested an increase in the nitric oxide cyclic guanosine-mono-phosphate pathway[18]. Thus , the regulation of naturally occurring inhibition of nitric oxide synthase activity could play an important role in control of PAR and hence blood pressure in normal PREG. Finally, increased placental blood flow functioning as an artero-venous shunt will aid further erosion of the PAR, MAP and pressure gradient of PREG.

The current result also revealed a reduction in TPP in the pregnant subjects starting early in PREG. This is suggestive of a possible PREG induced protein turnover especially in view of the elevated hormonal level and the rate of fetal growth which

TABLE 1

Anthropometric Measurements and Obstetric History of the Study Subjects

Variable	Group[n]		
	No pregnancy (control)	Pregnancy < 20wk	Pregnancy ≥ 20wk
Age (yr)	26.7 ± 2.90[†]	29.1 ± 1.30[ns]	29.4 ± 1.20[ns]
Height (m)	1.60 ± 0.01	1.61 ± 0.02[ns]	1.63 ± 0.01[ns]
Weight (kg)	85.0 ± 3.30	66.2 ± 2.90[*,#]	68.5 ± 3.60[*,#]
BMI (kg/m^2)	33.2 ± 2.30	25.6 ± 1.60[*]	25.8 ± 1.60[*,#]
Parity (n)	0-4	0-4[ns]	0-5[ns,#]

Values of anthropometric and obstetric determinations in pregnant (PREG) and no PREG (Control) subjects. [n] number of subjects per group = 25; [†] mean ± SEM; [ns] not significant compared with Control; [#] not significant compared with versus Pregnancy < 20wk; *p < 0.05 compared with Control. BMI, bodymass index.

TABLE 2

Blood pressure profile of the study subjects

Variable	Group[n]		
	No pregnancy (control)	Pregnancy < 20wk	Pregnancy ≥ 20wk
SBP	117 ± 2.2[†]	104.8 ± 2.5[*]	110.6 ± 2.3[*,ns]
DBP	80.3 ± 2.5	63.2 ± 1.8[*]	69.5 ± 2.3[*,#]
MAP	92.5 ± 2.2	77.06 ± 1.1[*]	83.2 ± 2.3[*,**]

Blood pressure profile in pregnant (PREG) and no PREG (Control) subjects. [n] number of subjects per group = 25; [†] mean ± SEM; *p<0.05 compared with Control; **p< 0.05 versus PREG < 20wk; [ns] not significant compared with Control; [#] compared with Pregnancy < 20wk. SBP, systolic blood pressure (mm Hg); DBP, diastolic blood pressure (mm Hg); MAP, mean arterial blood pressure (mm Hg).

TABLE 3

Hematological parameters of the study subjects

Variable	Group[n]		
	No pregnancy (control)	Pregnancy < 20wk	Pregnancy ≥ 20wk
Hct %	36.9 ± 0.6[†]	31.7 ± 0.6[*]	33.6 ± 0.4[*,#]
Hb (g/dl)	11.9 ± 0.4	10.8 ± 0.3[ns]	11.0 ± 0.5[ns, #]
Wbc (x 10^9/L) Total	5.4 ± 0.4	7.5 ± 0.5[*]	8.7 ± 0.3[*,**]
Platelet [x]10^9/L	246.7 ± 35.7	160.1± 13.2[*]	143.5± 13.2[*,**]
TPP g/dl	76.9± 3.2	73.5 ± 2.3[*]	71.5 ± 2.8[*,#]

Values of hematological parameters in pregnant (PREG) and no PREG (Control) subjects. [n] number of subjects per group = 25; [†]mean ± SEM; *p<0.05 compared with Control. **p<0.05 compared with Pregnancy<20wk; [ns] not significant compared with Control; [#]not significant compared with Pregnancy<20wk. Hct, hematocrit; Hb, hemoglobin; Wbc, white blood cell count; TPP, total plasma protein.

TABLE 4

Relative plasma and blood viscosity and RBC Deformability index[#] in study subjects

Variable	Group[n]		
	No pregnancy (control)	Pregnancy < 20wk	Pregnancy ≥ 20wk
RPV	1.88 ± 0.06[†]	2.35 ± 0.19[*]	2.65 ±0.o8[*,**]
RBV	6.80 ± 0.40	4.60±0.10[*]	4.30 ± 0.30[*,**]
DI[#]	3.62±0.40	1.96±0.20[*]	1.62 ±0.16[*,**]
DI as % of Control	100±5.0	54.0±5.0[*]	45.0±4.0[*,**]

Hemorheological indices in pregnant (PREG) and no PREG (Control) subjects. [n] number of subjects per group=25; [†] mean ± SEM;*p<0.05 compared with no Pregnancy Control; *p<0.05 compared with Pregnancy<20wk. RPV, relative plasma viscosity; RBV, relative blood viscosity; DI, RBC Deformability index = corrected blood viscosity (RBV)÷ corrected plasma viscosity (RPV)[10]

TABLE 5

The Pearson correlation coefficient (r) for blood pressure profile and homorheological factors with gestational factors with gestational age.

variables	pregnancy(<20 wks)	pregnancy(⩾20 wk)	p-level
DBP	0.49	0.41	p<0.001
SBP	0.45	0.43	p<0.025
MAP	0.48	0.51	p<0.05
Maternal Age			
WBC	0.72	0.86	p<0.001
RPV			
BPV	0.46	0.44	p<0.025

generally increases markedly especially in early PREG[2]. Similarly, both Hct and platelet count were decreased, accompanied by an elevated Wbc count in the studied pregnant subjects. The elevated Wbc count persisted into latter portion of PREG (PREG≥20wk) These findings are in tandem with views in literature that Hct, platelet count, Wbc count as well as plasma and blood viscosity each remains significantly associated with incident of coronary heart disease[20], towards which normal PREG is potentially predisposed[3].

The HH indices of the current study subjects exhibited a GA bias. The RPv was significantly elevated with diminished RBv especially in the late PREG (PREG≥20wk)There is currently no consensus on HH mechanism/dynamics of PREG. Whether erythrocyte deformability increases; falls, or remains unchanged during PREG is still to be confirmed. It is generally believed though, that in the first half of PREG the whole blood viscosity is maintained by the elevated plasma viscosity. As the Hct falls however, the plasma viscosity declines with a concomitant decline in whole blood viscosity[5,20].

In conclusion, the HH changes observed in this study are of advantage to the hyper-dynamic circulation of PREG. They help to explain the alteration in PAR and arterial blood pressure during normal PREG. Early in PREG, there is a reduction in general vasotone and a fall in blood viscosity. These changes putatively result in a reduction in PAR with a secondary fall in BP; and thereby help in averting the potential progression of PREG-induced CAD risk onto frank CAD.

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