**Stress hormones released pattern in blood donors and its impact on blood quality**

**Abstract**

**Background and objective**: The release of stress hormones in the course of blood donation varies according to the characteristics of the donor and may render red cells vulnerable to haemolysis. This study accessed the variation in donor characteristics on stress hormones release pattern and it influence on blood quality.

**Materials and method:** Serum levels of cortisol, adrenaline, noradrenaline, were measured and association analysis with haemolytic markers (Lactate dehydrogenase A (LDH-A), free plasma haemoglobin, osmotic fragility test) assessed in 252 whole blood donors. Beside osmotic fragility that employed Dacie’s method, these markers were evaluated with Melsin ELISA tests techniques.

**Results:**Variation in donor characteristicsaffected catecholamines (adrenaline and noradrenaline) levels but not cortisol. Adrenaline values were predominant in male (p = 0.012), replacement donors (p = 0.009) and donors exceeding 10 minutes bleed duration (p = 0.02). Still, differences were observed with noradrenaline levels in replacement (p = 0.004), first time (p = 0.03), the non-counselled (p = 0.03) and donors with no knowledge of blood donation (p = 0.02). Significant associations were observed between stress hormones and haemolytic markers. Adrenaline influenced LDH-A (p = <0.001), free plasma haemoglobin (p = <0.001), and red cell osmotic fragility test (p = 0.05). Similar trends were observed in cortisol with plasma hemoglobin (p = <0.001) and LDH-A (p = 0.03), while noradrenaline affected plasma hemoglobin (p = <0.001).

**Conclusion:** Variation in donor characteristics caused fluctuation on adrenaline, noradrenaline but not with cortisol. Stress hormones predisposed red cell to haemolysis hence, low quality blood product. These may have detrimental implications on donor safety and inefficient transfusion outcome.

**Keywords:** blood donor, blood quality, haemolytic markers, stress hormone.

**INTRODUCTION**

The primary goal in transfusion medicine is to provide blood of the required quality for safe and effective transfusion treatment. Blood products safety is of great concern worldwide, with World Health Organization promoting efforts to access to safe transfusion and safe blood products. Despite many initiatives and interventions, blood safety remains an important public health concern in Africa and other parts of the world where lack of availability of blood or provision of unsafe blood impacts morbidity and mortality (1). Safety of blood products begins with blood donor recruitment (2). Though the altruistic gesture brings positive feeling to blood donors, blood donation is capable of inducing psychological, hormonal or physiological stress responses (3–5), that may temper with donors return rate and blood quality.

When transposed to a blood donation setting, the donation itself, or each step in a donation procedure, might elicit fear accompanied with stress hormones release(6). This hormones may play an important role in the fragility and deformability of red blood cells (7–9). The consequences of fragile red cell membrane is hemolysis (10) while deformability resistance increases blood capillary resistance for RBCs passage to the body cells for carrying normal metabolism and may lead to toxicity in the recipient (11).

The magnitude of this response is donor dependent (12). Variability in blood donor population impact blood quality, as the characteristics of the donor is reflected in the biochemistry of an individual blood pint (13). This in turn may affect transfusion efficacy and patient outcomes (13). Several factors influencing stress response include but are not limited to age, sex, personality, physical and mental health, donor’s experiences (14), number of donations.

The physiology of stress response has 2 components: A slow response mediated by the HPA axis and a fast response mediated by the SAM axis. The quick response triggered by SAM activation leads to increased secretion of norepinephrine and epinephrine from the adrenal medulla and from the sympathetic nerves, resulting in serum elevated levels (15). Evidence also points to the involvement in the autonomic response to stress through increased secretion of catecholamines (16).  The released epinephrine and norepinephrine interact with α- and β-adrenergic receptors in the central nervous system and on the cell membrane of smooth muscles resulting in the contraction of smooth and cardiac muscle cells (17). Vasoconstriction, increased blood pressure, heart rate, cardiac output, skeletal muscle blood flow, increased sodium retention, increased levels of glucose (glycogenolysis and gluconeogenesis), lipolysis, increased oxygen consumption, and thermogenesis as consequences(17). In long term stress response, the burst of energy is mediated greatly by cortisol from the adrenal cortex triggered by hypothalamus through the release of ACTH from the anterior pituitary. Cortisol primarily affect glucose metabolism by stimulating glucose synthesis and breakdown fat into fatty acids for other tissues to use for ATP production (18).

Erythrocytes are circulating pools for cortisol and constitute both targets and transporters of various hormones, as studies report erythrocytes response to signals initiated after binding to epinephrine, and norepinephrine (19). Signaling results in regulation of cellular metabolism and membrane fluidity (19). This interaction is possible thanks to the presence of beta-adrenergic receptors (β-ARs) found on red cell membrane that are physiologically activated by epinephrine and norepinephrine(20).

The interaction causes structural changes in the red blood cell, affecting its mechanical properties, thus leading to changes in the blood flow in microcirculation with impaired tissue perfusion(7). So far studies on hormonal stress response in blood donation process centres on cortisol (5,21,22) with little known on other stress hormones (adrenaline and noradrenaline).

In this study, we assessed the pattern of stress hormones released in relation to variation in donor characteristics and defined its impact on red cell quality. Based on the hypothesis that, the release of stress hormones in the course of blood donation varies according to the donor’s characteristics and may render red cells vulnerable to haemolysis, thereby lowering the quality of blood products.

**MATERIALS AND METHODS**

**Study Design and Sampling**

Data used for this study was gathered from a 24 month experimental study carried out at the Bafoussam Regional hospital blood bank (BRHBB), West Region of Cameroon. Participants consisted of counselled and non-counselled eligible blood donors. Sampling was done by a random pick up of number found in a ballot box with a match of category of donor, number of donations and treatment intervention (counselled and non-counselled) as shown in Table 1.

**Table 1: Variables of the study population stratified by treatment intervention**

|  |  |  |  |
| --- | --- | --- | --- |
| Stratum  | Counselled | Non-counseled  | Total (%) |
| Female Voluntary 1st donation | 7 | 7 | 14 |
| Female Replacement 1st donation | 5 | 7 | 12 |
| Female Voluntary >1 donation | 7 | 6 | 13 |
|  Female Replacement >1 donation | 7 | 6 | 13 |
| Male Voluntary 1st donation | 23 | 23 | 46 |
| Male Replacement 1st donation | 26 | 28 | 54 |
| Male Voluntary >1 donation | 23 | 22 | 45 |
|  Male Replacement >1 donation | 27 | 28 | 55 |
| Total | 125 | 127 | 252 |

**Study Population and** **Data Collection Procedure**

Consented eligible whole blood donors of age 18-65years free from hemolytic pathologies and medication and who turn up before 10 a.m. at the BRHBB for donation were enrolled. Information on socio-demographic profile (age, sex, level of educational level) and blood donation history (type of donor, number of donations, ADR previous experience) was gathered using a structured questionnaire. Sample for biochemical investigations of stress hormones and hemolytic markers was collected by deviating a 10ml blood sample into a 5ml dry and 5ml EDTA vacuum tube at the donation stage.

One-step double-antibody sandwich MELSIN ELISA kits for human cortisol, adrenaline, noradrenaline, plasma hemoglobin and LDH-A were employed for measurements. The principle was the same for the above mentioned markers. 50 μL of standards and 10 μL of samples were pipetted into appropriate wells containing antibodies of above mentioned markers in the micro Elisa strip plate. 40 μL of diluent added to sample wells (sample diluted 5-fold). Followed by the addition of 100 μL of HRP-conjugate reagent into each well, gently shake, cover with adhesive strip and incubated for 1 hour at 37oC. The strip plate washed 5 times with an ELx50TM BioTek microplate strip washer and blot against a clean paper towel. 50 μL each of chromogen A and B were added to each well, mixed and incubated for additional 15minutes at 37oC minutes. 50 μL of stop solution was added to each well and the optical density read on ELx800TM BioTek absorbance microplate reader at 450 nm. The concentrations were determined from a standard curve plotted using standard concentrations with different optical density values. To obtain the real measurements, the concentrations read from standard curve were multiplied by the dilution factor (5).

Erythrocyte osmotic fragility test was analyzed within 2hours of sample collection by Dacie,s method. RBCs were placed in freshly prepared serial solutions of saline at concentrations ranging from 0 % to 0.88 % NaCl (table 2) and hemolysis evaluated.

The 12 test tubes numbered serially from tube 1 to tube 12. Drops of distilled water dispensed into it at different volumes. Later, drops of freshly prepared 1% saline solution added in different volumes. This was followed by dropping in each of the 12 tubes a drop of homogenized whole blood. Tubes were gently shake to avoid mechanical hemolysis and then allow to stand for an hour. Hemolysis evaluation was qualitative and quantitative. Qualitatively, visual readings were done by observing which tube experienced onset and completion of hemolysis. Quantitatively, the were centrifuged at 2000rpm for 2 minutes.100ml of the supernatants transferred into titration wells and hemoglobin concentrations determined spectrophotometrically by ELx800TM BioTek absorbance microplate reader at 540 nm within 15 minutes.

**Table 2: Osmotic fragility testing procedure**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test tube No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Distilled H2O drops Vol (µl) | 3 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 25 |
| 150 | 450 | 500 | 550 | 600 | 650 | 700 | 750 | 800 | 850 | 900 | 1250 |
| 1% saline (drops)  Vol (µl) | 22 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 0 |
| 1100 | 800 | 750 | 700 | 650 | 600 | 550 | 500 | 450 | 400 | 350 | 0 |
| Tonicity (g/dL) | 0.88 | 0.64 | 0.60 | 0.56 | 0.52 | 0.48 | 0.44 | 0.40 | 0.36 | 0.32 | 0.28 | 0 |
| Blood sample  drops  Vol (µl) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |

Analysed were with Epi Info version 7.2.2.6 computer software. Every analysis was done at 95% confidence interval. Risk ratios & Chi-square (χ2) tests were used to compare socio-demographic characteristics and identified significant associations. Means and student t-tests were employed in comparison of groups in the studied population. P-Values less than 0.05 were considered statistically significant.

Sample size calculated using the formula, n = log β/log p (Dell et al)

β = probability of committing a type 11 error (0.05)

p = proportion of donors with AR. 2.8% (97.2 without adversity)

n = log 0.05/ log o.972

 n = 106

Minimum required per group 106

**Ethical considerations**

The study protocol was approved by Institutional Review Board of the Faculty of Sciences, University of Buea, Cameroon (Ref: 2020/1244-04/UB/SG/IRG/FHS), and administratively, an authorisation delivered by the Director of the BRH.

**RESULTS**

Up to 252 whole blood donors, 125 counselled and 127 non-counselled were recruited from 16 strata. The minimum participants per stratum and per gender were 5 and 22 for female and male respectively (Table 1).

Of the 252 donors, 200 (79.4%) were of male gender (Table 3). The results of the analysis revealed no significant associations between cortisol levels (low, high, and normal) and the sub-variables studied, including gender (p=0.27), donor type (p=0.80), number of donations (p=0.45p), bleed duration (p=0.98), past history of adverse drug reactions (p=0.83), intervention (p=0.84), and knowledge of blood donation (p=0.94) (Table 3). Males constituted the majority across cortisol categories, and replacement donors were more common than voluntary donors; however, these distributions did not significantly influence cortisol levels. Similarly, the number of donations and bleed duration were evenly distributed across groups, with no measurable impact. Most donors reported no prior adverse reactions, and counselling and donor knowledge did not alter cortisol distributions (Table 3).

**Table 3: Donor characteristics and cortisol levels**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sub variable** | **Cortisol** | **Total (%)** | **χ2** | **p-Value** |
| Low (%)  | High (%) | Normal (%)  |  |  |  |
| **Gender** | Male | 93(80.87) | 52(83.87) | 55(73.33) | 200 | 2.593 | 0.27 |
| Female | 22(19.13) | 10(16.13) | 20(26.67) | 52 |
| **Donor type** | Replacement | 59(56.45) | 35(51.30) | 40(53.33) | 134 | 0.42 | 0.80 |
| Voluntary | 56(47.46) | 27(22.88) | 35(29.66) | 118 |
| **No of donations** | 1 | 61(53.04) | 32(51.61) | 33(44) | 126 | 1.57 | 0.45 |
| > 1 | 54(46.96) | 30(48.39) | 42(56) | 126 |
| **Bleed duration (minutes)** | ≤10 | 94(81.74) | 51(82.26) | 62(82.67) | 207 | 0.027 | 0.98 |
| >10 | 21(18.26) | 11(17.74) | 13(17.33) | 45 |
| **Past history of ADR** | No | 110(96.77) | 60(95.65) | 71(94>67) | 241 | 0.36 | 0.83 |
| Yes | 5(3.23) | 2(4.35) | 4(5.33) | 11 |
| **Intervention** | Counselled | 59(51.30) | 29(46.77) | 37(49.33) | 125 | 0.33 | 0.84 |
| Non counselled | 56(48.70) | 33(53.23) | 38(50.67) | 127 |
| **Knowledge of blood donation** | No  | 49(42.61) | 28(45.16) | 33(44) | 110 | 0.11 | 0.94 |
| Yes  | 66(57.39) | 34(54.84) | 42(56) | 142 |

Investigating the relationship between adrenaline levels (Normal, High, and Low) and some factors among blood donors revealed: significant associations between adrenaline levels and gender (p=0.012), donor type (p=0.009), and bleed duration (p=0.02) (Table 4). Gender showed a notable influence on adrenaline levels, with males predominantly falling in the normal and high adrenaline categories, while females were more represented in the low adrenaline group. Donor type also demonstrated a significant relationship, as voluntary donors were more likely to have high adrenaline levels, whereas replacement donors were more prevalent in the normal and low categories. Additionally, bleed duration was associated with adrenaline levels, with shorter donation times (≤10 minutes) being more common in the normal adrenaline group, and longer durations (>10 minutes) linked to both low and high adrenaline levels.

**Table 4: Donor characteristics and adrenaline**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sub variable** | **Cortisol** | **Total (%)** | **χ2** | **p-Value** |
| Low (%) | High (%) | Normal (%)  |  |  |  |
| **Gender** | Male | 86(71.67) | 13(92.86) | 101(85.56) | 200 | 8.69 | 0.012 |
| Female | 34(28.33) | 1(7.14) | 17(14.41) | 52 |
| **Donor type** | Replacement | 58(48.33) | 2(14.29) | 74(62.71) | 134 | 13,84 | 0,009 |
| Voluntary | 62(51.67) | 12(85.71) | 44(37.29) | 118 |
| **No of donations** | 1 | 59(49.17) | 10(71.43) | 57(48.37) | 126 | 2.74 | 0.25 |
| > 1 | 61(50.83) | 4(28.57) | 61(51.69) | 126 |
| **Bleed duration (minutes)** | ≤10 | 92(76.67) | 10(71.47) | 105(88,98) | 207 | 7.31 | 0.02 |
| >10 | 28(23.33) | 4(28.57) | 13(11.02) | 45 |
| **Past history of ADR** | No | 114(95) | 14(100) | 113(95.76) | 241 | 0.75 | 0.68 |
| Yes | 6(5) | 0(0) | 5(4.24) | 11 |
| **Intervention** | Counselled | 58(48.33) | 6(42.86) | 61(51.69) | 125 | 0.53 | 0.76 |
| Non counselled | 62(51.67) | 8(57.14) | 57(48.31) | 127 |
| **Knowledge of blood donation** | No  | 48(40) | 7(50) | 55(46.61) | 110 | 1.29 | 0.52 |
| Yes  | 73(60) | 7(50) | 63(53.39) | 142 |

Analyzing the relationship between noradrenaline levels (Normal, High, and Low) and some factors among blood donors revealed: significant associations with donor type (p=0.004), number of donations (p=0.03p), intervention (p=0.03), and knowledge of blood donation (p=0.02) (Table 5). Donor type showed a strong influence, with voluntary donors predominantly exhibiting high noradrenaline levels, while replacement donors were more prevalent in the normal and low categories. Similarly, the number of donations was significantly associated, as donors with multiple donations were more represented in the high and low noradrenaline categories, whereas first-time donors were primarily found in the normal category. Intervention (counselling) also affected noradrenaline levels, with a greater proportion of counselled donors showing high levels compared to non-counselled donors, who were more evenly distributed across categories. Moreover, knowledge of blood donation demonstrated a significant relationship, as donors with prior knowledge had higher noradrenaline levels compared to those without knowledge, who were more represented in the normal and low categories (Table 5).

**Table 5: Donor characteristics and noradrenaline levels**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variable**  | **Sub variable** | **Noradrenaline** | **Total (%)** | **χ2** | **p-Value** |
| **Normal (%)** | **High (%)** | **Low (%)** |  |  |  |
| **Gender** | **Male** | 64(83.12) | 21(80.77) | 115(77.18) | 200 | 1.12 | 0.56 |
| **Female** | 13(16.88) | 5(19.23) | 34(22.82) | 52 |
| **Donor type** | **Replacement** | 42(54.55) | 6(23.08) | 86(57.72) | 134 | 10.75 | 0.004 |
| **Voluntary** | 35(45.45) | 20(76.92) | 63(42.28) | 118 |
| **No of donations** | **1** | 48(62.34) | 11(42.31) | 67(44.97) | 126 | 6.81 | 0.03 |
| **> 1** | 29(37.66) | 15(57.69) | 82(55.03) | 126 |
| **Bleed duration (minutes)** | **≤10** | 63(81.82) | 21(80.77) | 123(82.55) | 207 | 0.05 | 0.97 |
| **>10** | 14(18.18) | 5(19.23) | 26(17.45) | 45 |
| **Past history of ADR** | **No** | 75(97.40) | 26(100) | 9(6.04) | 11 | 2.76 | 0,25 |
| **Yes** | 2(2.60) | 0(0) | 140(93.96) | 241 |
| **Intervention** | **Counselled** | 30(38.96) | 17(65.38) | 78(52.35) | 125 | 6.52 | 0.03 |
| **Non counselled** | 47(61.04) | 9(34.62) | 71(47.65) | 127 |
| **Knowledge of blood donation** | **No**  | 42(54.55) | 7(26.92) | 61(40.94) | 110 | 7.11 | 0.02 |
| **Yes**  | 35(45.45) | 19(73.08) | 88(59.06) | 142 |

Analyzing the relationship between hormone levels (adrenaline, cortisol, and noradrenaline) and the occurrence of adverse drug reactions (ADRs), the result indicates no statistically significant associations for any of the hormones evaluated (Table 6).

For adrenaline, the proportion of donors with ADRs was slightly higher in the high adrenaline group (21.43%) compared to the normal (14.41%) and low (12.50%) groups, but the risk ratio (RR: 1.4874, 95% CI: 0.4975–4.4472) and p=0.642 suggest no significant association (Table 6). Similarly, cortisol levels showed no significant relationship with ADRs (p=0.484). The proportion of donors with ADRs was comparable across normal (14.67%), high (17.74%), and low (11.30%) cortisol categories, with a risk ratio of 1.2097 (95% CI: 0.5629–2.5997) (Table 6). For noradrenaline, the prevalence of ADRs was highest in the normal group (16.88%) compared to the high (11.54%) and low (12.75%) groups, but the risk ratio (RR: 0.6834, 95% CI: 0.2113–2.2110) and p=0.65 confirm no significant association (Table 6).

**Table 6: The relationship between stress hormones levels and ADR**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hormone** | **ADR** | **Total**  | **RR (95%)** | **χ2** | **p-value** |
|  | Yes n (%)  | No n (%)  |   |   |   |   |
| **Adrenaline** |   |
|  Normal | 17(14.41) | 101(85.59) | 118 | 1.4874 (0.4975-4.4472) | 0.8852 | 0.642 |
|  High | 3(21.43) | 11(78.57) | 14 |
|  Low | 15(12.50) | 105(87.50) | 120 |
| **Cortisol** |   |
|  Normal | 11(14.67) | 64(85.33) | 75 | 1.2097(0.5629-2.5997) | 1.449 | 0,484 |
|  High | 11(17.74) | 51(82.26) | 62 |
|  Low | 13(11.30) | 102(88.70) | 115 |
| **Noradrenaline** |   |
|  Normal | 13(16.88) | 64(83.12) | 77 | 0.6834(0.2113-2.2110) | 0.584 | 0.65 |
|  High | 3(11.54) | 23(88.46) | 26 |
|  Low | 19(12.75) | 130(87.25) | 149 |

**Stress hormones levels and blood quality**

Our evaluation on the association between stress hormones and red cell quality markers revealed that; as adrenaline and cortisol levels increase, so do LDH-A , with (p = <0.001) and (p = 0.03) respectively. Still, significant associations were found between adrenaline (p = <0.001), cortisol (p = <0.001) noradrenaline (p = <0.001) and plasma hemoglobin (table 7).

**Table 7: Stress hormones and Lactate dehydrogenase-A**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hormone**  | **LDHA** | **Total**  | **RR (95%)** | **χ2** | **p-Value** |
| Normal(%) | High (%)  | Low (%) |
| **Adrenaline** |  |  |  |  |  |  |  |
| **Normal** | 54(45.76) | 5(4.24) | 59(50) | 118 | 8.4286(3.4212-20.7651) | 23.74 | **< 0.001** |
| **High** | 4(28.57) | 10(71.43) | 0(0) | 14 |  |  |
| **Low** | 35(29.17) | 4(3.33) | 81(67.50) | 120 |  |  |
| **Noradrenaline**  |  |  |
| **Normal** | 39(41.94) | 7(7.53) | 47(50.54) | 93 | 2.52275 (0.9595-6.6577 | 3.37 | 0.06 |
| **High** | 8(42.11) | 5(26.32) | 6(31.58) | 19 |
| **Low** | 30(21.43) | 14(10) | 96(68.57) | 140 |
| **Cortisol**  |  |  |  |  |  |  |  |
| **Normal** | 33(44) | 3(4) | 39(52) | 75 | 4.7143(1.2079-18.3994 | 4.330 | **0.03** |
| **High** | 28(45.16) | 12(19.35) | 22(35.58) | 62 |  |  |  |
| **Low** | 32(27.83) | 4(3.48) | 79(68.70) | 115 |  |  |  |

The relationship between plasma hemoglobin levels (Normal, High, and Low) and hormone levels (adrenaline, cortisol, and noradrenaline) was analyzed, revealing significant associations across all three hormones (Table 8).

For adrenaline, plasma hemoglobin levels were strongly associated (p<0.001). In the normal adrenaline group, a higher proportion of donors exhibited low plasma hemoglobin (52.54%), while fewer had normal (32.20%) or high (15.25%) levels (Table 8). The high adrenaline group showed a greater proportion of donors with high plasma hemoglobin (50%), while the low adrenaline group predominantly exhibited low plasma hemoglobin (70%) (Table 8).

Cortisol also demonstrated a significant relationship with plasma hemoglobin levels (p<0.001). In the normal cortisol group, low plasma hemoglobin levels were the most common (60%), while normal (28%) and high (12%) levels were less frequent. Donors in the high cortisol group were more evenly distributed across normal (27.42%), high (38.71%), and low (33.87%) plasma hemoglobin levels. The low cortisol group showed a predominance of low plasma hemoglobin (70.43%) (Table 8).

For noradrenaline, the association with plasma hemoglobin levels was similarly significant (p<0.001). In the normal noradrenaline group, low plasma hemoglobin levels were the most frequent (35.06%), followed by normal (37.66%) and high (27.27%) levels. Donors in the high noradrenaline group exhibited higher plasma hemoglobin levels (42.31%), while those in the low noradrenaline group were predominantly in the low plasma hemoglobin category (75.84%) (Table 8).

**Table 8: Stress hormones and plasma hemoglobin**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hormone**  | **LDHA** | **Total**  | **RR (95%)** | **χ2** | **p-Value** |
| Normal(%) | High (%)  | Low (%) |
| **Adrenaline** |  |  |  |  |  |  |  |
| **Normal** | 38(32.20) | 18(15.25) | 62(52.54) | 118 | 1.6752(0.8913-3.1484) | 27.80 | **<0.001** |
| **High** | 6(42.86) | 7(50) | 1(7.14) | 14 |  |  |
| **Low** | 23(19.17) | 13(10.83) | 84(70) | 120 |  |  |
| **Cortisol** |  |  |  |  |  |
| **Normal** | 21(28) | 9(12) | 45(60) | 75 | 1.6752(0.8913-3.1484) | 41.64 | **<0.001** |
| **High** | 17(27.42) | 24(38.71) | 21(33.87) | 62 |
| **Low** | 29(25.22) | 5(4.35) | 81(70.43) | 115 |
| **Noradrenaline**  |  |  |  |  |  |  |  |
| **Normal** | 29(37.66) | 21(27.27) | 27(35.06) | 77 | 1.2440(0.4133-3.7551) | 57.874 | **<0.001** |
| **High** | 8(30.77) | 11(42.31) | 7(26.92) | 26 |  |  |  |
| **Low** | 30(20.13) | 6(4.03) | 113(75.84) | 149 |  |  |  |

Evaluating the relationship between hormone levels (adrenaline, cortisol, and noradrenaline) and erythrocyte osmotic fragility status (non-fragile vs. fragile) among donors.

For adrenaline, there was a significant borderline association with osmotic fragility test (OFT) (p=0.05). In the normal adrenaline group, the majority of donors (78.81%) exhibited non-fragile erythrocytes, while 21.19% were fragile (Table 9). Similar distributions were observed in the high adrenaline group (78.57% non-fragile, 21.43% fragile). However, in the low adrenaline group, a higher proportion of donors showed fragile erythrocytes (35%) compared to the other groups (Table 9).

Noradrenaline did not show a significant association with OFT (p=0.22). In the normal noradrenaline group, 64.84% of donors had non-fragile erythrocytes, while 35.06% were fragile. The high noradrenaline group had a higher percentage of non-fragile erythrocytes (73.08%) compared to fragile (26.92%). In the low noradrenaline group, most donors (75.84%) were non-fragile, with 24.16% being fragile.

For cortisol, no significant association with OFT was observed (p=0.29). In the normal cortisol group, 74.67% of donors had non-fragile erythrocytes, compared to 64.52% in the high cortisol group and 74.78% in the low cortisol group (Table 9). Fragile erythrocytes were more frequent in the high cortisol group (35.48%) than in the normal (23.33%) or low (25.22%) groups (Table 9).

**Table 9: Stress hormones level with respect to fragile red cells**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hormone**  | **OFT** | **Total** | **RR (95%)** | **χ2** | **p-Value** |
|  **Non-fragile (%)**  | **Fragile (%)**  |  |  |  |  |
| **Adrenaline** **Normal****High** **Low**  |   |   |
| 93(78.81) | 25(21.19) | 118 | 1.0114(0.3498-2.9244) | 5.95 | **0.05** |
| 11(78.57) | 3(21.43) | 14 |
| 78(65) | 42(35) | 120 |
| **Noradrenaline****Normal** **High** **Low**  |   |   |
| 50(64.84) | 27(35.06) | 77 | 0.6823(0.2548-1.8269) | 2.019 | 0.22 |
| 19(73.08) | 7(26.92) | 26 |
| 113(75.84) | 36(24.16) | 149 |
| **Cortisol** **Normal** **High****Low**  |   |   |
| 56(74.67) | 19(23.33) | 75 | 1.6211(0.7767-3.3835) | 2.43 | 0.29 |
| 40(64.52) | 22(35.48) | 62 |
| 86(74.78) | 29(25.22) | 115 |

**Discussion**

Variation in blood donor characteristics may influence stress hormones released pattern, thereby modulating the quality of blood products with compromised transfusion efficacy as consequences.

Our study portrays how serum levels of adrenaline and noradrenaline but not cortisol were affected by donor characteristics. A similar study on hormonal adaptation to blood donation indicated higher plasma concentrations of catecholamines following blood donation (23). The reason may recite on the fact that, the normal bleed duration for a 450ml pint of whole blood is between 6 to 10 minutes(24). An interval that permits the release of adrenaline and noradrenaline with a 2minutes serum release in stress response compared to cortisol with 15 minutes (25–27).

We observed gender, donation status and bleed duration influenced on adrenaline fluctuations. The females folk (p= 0.012), voluntary donors (0.009) and donors bled within 10 minutes (p = 0.02) presented low adrenaline levels. Frankenhaeuser and his collaborators studied sex difference in stress induced pattern of adrenaline excretion and reported males inducing a significant increase (28). Female vulnerability to low levels of adrenaline carries several attributions. Initially, women have the same response to stress as men, leaving them somewhat vulnerable to cortisol and adrenaline. But the secretion of oxytocin in female, scale back the production of cortisol and adrenaline. Though, men secrete oxytocin under stress, but production is lesser, coupled with the fact that its effects are inhibited by male hormones (testosterone)(29). Furthermore, the Y-chromosome gene SRY controls a group of neurotransmitters (catecholamines) by exerting male-specific effects in tissues outside the testis, thereby priming organs in males to respond to stress through increased release of catecholamine which drive fight-or-flight in males(30). Voluntary donors are recognised as the safest group as they are regularly screened for TTI and are psychologically motivated by altruism to willingly donate without constraints(31). For that reason, they may exhibit low adrenaline levels with respect to replacement donors. Our previous studies revealed prolonged bleed duration as a predisposing factor of adverse donor reaction. Still the present study portrays a positive association between adrenaline on prolonged bleed duration. This necessitate further findings.

Similar noradrenaline release pattern were significantly low with the non-counselled (p=0.03), voluntary (p=0.004), first donations (p=0.03) status as well as in donors who have poor knowledge (p=0.02) in blood donation activities. Apart from voluntary donors, a common characteristics found amongst these groups of donors with low levels of noradrenaline is the aspect of non-familiarity to blood donation services. Donors with high levels adrenaline and cortisol were more likely to develop ADR at a relative risk of 1.5 and 1.2 respectively. Elevated cortisol and adrenaline levels can exacerbate anxiety, increase blood pressure, narrow blood vessels and compromise blood supply to vital organs and as a result distress, dizziness, light headache(32) during the blood donation. Whether a donor was counselled or not, no statistical difference was found on pattern of release of cortisol, adrenaline and noradrenaline.

The hypothesis that stress hormones impact blood quality, could be supported by the fact that cortisol, adrenaline and noradrenaline were associated to haemolysis as observed in haemolytic markers; LDH-A, free plasma haemoglobin, and OFT. Significant serum LDH-A levels were found in adrenaline (p=<0.001) and cortisol (p=0.03). This is confirmed by Cui and collaborators who demonstrated a positive association between high adrenaline in patient serum and high LDH-A (33). Elevated LDH in serum is as a consequence of organ destruction resulting in loss of cytoplasmic content (34). Stress-induced epinephrine enhances LDH-A-dependent metabolic activity(33). In the same light, blood donors experiencing stress, exhibit adrenaline rush with increase LDH-A for glycolysis (35). Findings in India, revealed stressed group exhibiting higher levels of cortisol and LDH (36). Similar trends of increase plasma hemoglobin concentration were observed in high levels of adrenaline (p=<0.001), cortisol (p=<0.001) and noradrenaline (p=<0.001). Haemoglobin is contained within erythrocyte. Normal blood draw procedures caused limited unavoidable disruption and small amount may normally be present. A significant amount in plasma is an indication of RBC disruption for any reason amongst which are stress hormones (33). Metabolic abnormalities including catecholamines developing during stressful conditions may actively trigger, accelerate or amplify hemolysis with free plasma hemoglobin released (37). Further, we assessed the link between stress hormones and osmotic fragility test, and found an association with adrenaline (p=0.05). HPA endocrine metabolic abnormal substances (catecholamine) released by donation stress may induce haemolysis(37). The mechanism of stress hormones inducing haemolysis may be justified by the fact that, adrenaline and noradrenaline are receptor ligands to alpha and beta-adrenergic receptors expressed on the membrane of erythrocyte(38). Their interaction renders red cell membrane sensitive to vasoactive substance(7), causing impaired membrane deformability and vulnerability to hemolysis (39). Stress hormones (cortisol, adrenaline, noradrenaline) binding to erythrocyte membrane involves hydrogen, hydrophobic & electrostatic interactions. Active groups of these hormones [NH(2), NHCH(3), keto & hydroxyl groups] interact with CO & NH groups both of protein and phospholipids leading to formation of complex protein domain that distorts red cell membrane (tangle α helix & β structure) facilitating loosening and deteriorates elasticity/rheological properties (40).

If metabolic causes and mechanisms enhanced erythrocyte fragility and/or impaired membrane structure, deformability resistance, intravascular destruction (37), what then is the transfusion outcome with such blood pints.

On investigating whether there exists a difference between red cell quality markers amongst the counselled and the non-counselled group, there was no variation in concentration of LDH-A plasma hemoglobin, no an influence on red cell fragility test.

**Conclusion**

This study highlights strong associations between plasma hemoglobin levels and all three hormones (adrenaline, cortisol, and noradrenaline), suggesting that hormonal variations may significantly influence hemoglobin status. Adrenaline levels showed a borderline significant relationship with erythrocyte osmotic fragility, with increased fragility observed in donors with low adrenaline levels. These findings also suggest that adrenaline may influence erythrocyte stability, warranting further investigation into its physiological effects in donors. Across all three hormones, the analysis did not find significant differences in the occurrence of ADRs among the groups. These findings suggest that variations in hormone levels (adrenaline, cortisol, and noradrenaline) are not predictive of ADRs in this donor cohort, highlighting the need for further investigation into other factors influencing ADR risk during blood donation. Further research is needed to explore the mechanisms underlying these relationships and their clinical implications for donor physiology and blood donation practices.

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