Original Article

Leukocyte Profile of Adult Nigerian Subjects with Acute Musculoskeletal Trauma

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ABSTRACT

BACKGROUND: Leukocyte levels are evidently useful in trauma assessment, prognostication and management.[1] Leukocytosis is a known physiologic response to trauma[2] but suggested to be absent among Africans origin[3]. The aim of present study is to investigate the existence of leukocytosis among adult Nigerians who sustain acute musculoskeletal trauma, and also assess its prognostic value in management.

METHODS: A calculated sample size of 223 subjects participated following informed consent while 50 apparently healthy adult Nigerian volunteers served as control. Data obtained included age, sex, duration of hospitalization and leukocyte counts using improved Neuabauer chamber while differential count was done on stained thin film.

RESULTS: Analyzed results expressed as mean $\pm$ SDM show significant male dominance ($p < 0.05$) among the acutely traumatized subjects with majority (70%) aged between 20 and 49 years. The mean total white blood cell count was 8184.96 $\pm$ 201.087 cells/mm^3, significantly higher than mean for the control group (4922.00+1282.264 cells/mm3) at $p < 0.05$. The mean values for neutrophil and lymphocytes also show significant neutrophilia and lymphocytopaenia among the test population ($p < 0.05$). Pearson's correlation test for duration of hospitalization was positive for higher levels of total White Blood Cell count, with strong positive correlation between the levels of neutrophil and total White Blood Cell count suggesting the source of the observed leukocytosis.

CONCLUSION: Thus adult Nigerians exhibit post-traumatic neutrophilic leukocytosis contrary to some widely accepted postulations. The post-traumatic neutrophilia exhibit an inverse relationship with observed lymphocytopaenia. This can be expressed as a ratio called Neutrophil Lymphocyte Stress Factor (NLSF) and used in trauma management and prognosis determination.

KEYWORDS: Leukocyte profile, Adult Nigerians, Acute musculoskeletal trauma

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INTRODUCTION

There is consensus of opinion among researchers that Africans are leukopaenic relative to Caucasians[4,5,6]. This leukopaenia does not seem to affect the occurrence of leukocytosis following acute infection in subjects of African origin[3]. Acute Leukocytosis is also known to be part of the body's metabolic response to trauma directed towards containing the effect of trauma of any sort, thereby restoring homeostasis[2]. The commonest cause of trauma in our environment is road traffic accidents, gunshot wounds and falls from height[7]. Traumatic injuries include fractures of bones, muscular injuries, tendon and ligamentous ruptures or sprains etc. The musculoskeletal system constitute over 70% of the entire human body hence the likelihood of its affectation is very high any time there is incidence of trauma.

There is limited information on the pattern of leukocyte response of Nigerians to acute musculoskeletal injury. This is important as it may have prognostic implications as suggested by Kho *et al*, that moderate leukocytosis is required for good prognosis following musculoskeletal trauma while high absolute lymphocytosis, leukocytosis and leukopaenia is associated with increased morbidity and mortality[1]. There is also consistent increase in circulating free radicals post trauma mainly due to increased polymorphonuclear (PMN) leucocytes mobilized acutely[8] . Muster *et al* while studying activation of blood coagulation in pigs following lower limb trauma noted an increased creatine kinase, body temperature, metabolic and respiratory alkalosis as well as moderate leukocytosis[9]. The source of leukocytosis following trauma has been established to include mobilization of marginated leucocytes, stimulation of bone marrow by cytokines e.g. tumour necrosis factor (TNF), interleukins-1, 6, and 8, Nitric oxide, platelet activation factors (PAF) etc. These and other mediator molecules are products of cascade of events involving lipopolysaccharides from damaged tissues and molecules from leucocytic degranulation[10] . Laing *et al* were able to demonstrate definite two fold increase in leucocyte level within 48 hours of closed fracture of the femur in mice while studying the response of bone marrow to musculoskeletal trauma[11].

Strenuous exercise is also known to produce peripheral leucocytosis acutely. Sodique et al were able to categorically demonstrate exercise-induced leucocytosis in healthy adult Nigerians[22]. They concluded that the marked leucocytosis was mainly due to lymphocytosis, demargination of leucocytes and influx of lymphocytes into the circulation from lymphatic vessels. This is so because during exercise the body mobilizes blood from the areas of physiologic reserves of the viscera into the circulation to increase the cardiac output.

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Post-traumatic leucocytosis is not restricted to adults as a study of peripheral blood picture of children with head trauma showed an increase in total white blood cell count, neutrophil and band counts[12]. Similarly, absolute leucocytosis and neutrophilia demonstrated within 48 hours of cardiopulmonary bypass surgery cut across sex and age[13]. In the elderly, these responses to trauma are present but are limited and modified by poor physiologic reserves and co-morbid medical conditions[14].

Consistent leukocytosis following acute trauma has been reported on Caucasian populations and animal models, but there is dearth of information in literature on black populations living in Africa and their leukocyte response to trauma. To scientifically prognosticate using this parameter in traumatized Africans will be more meaningful if such leukocytosis or otherwise is confirmed in our population considering the significant variation in blood parameters of Africans and Caucasians[5].

MATERIALS AND METHOD

This cross sectional study was carried out at National Orthopaedic Hospital Enugu, a regional centre for trauma, orthopaedic, burns and plastic surgery. Adult Nigerians aged 18 years and above who sustained acute musculoskeletal trauma through road traffic accidents or clean elective orthopaedic operations were recruited. Those with open fractures and spinal cord injuries were excluded. A total of 223 subjects and 50 apparently healthy adult Nigerian volunteers were also recruited as the control group. The hospital number, age, sex and injury or surgery of each participant were recorded and venous blood collected from the forearm and then stored in an EDTA bottle. Total white blood cell counts were obtained using the Improved Neubauer Heamocytomer. The differential counts (neutrophil, lymphocyte, basophil, eosinophil and monocyte) were obtained by microscopic examination of blood films using Leishman's stained blood smear method[35] (Daci and Lewis, 1994). Results were expressed as mean $\pm$ SDM with level of significance set at $p < 0.05$. Levene's t-test and Analysis of variance test (ANOVA) were used to access the effect of sex and age respectivel.

RESULTS

Table 1 shows male dominance (76.4%) of the subjects with acute musculoskeletal trauma with male to female ratio was 4:1 for this test group and 3:2 for the control . There was significant age variation in the incidence of trauma as age distribution of the post traumatic subjects with majority (72%) aged between 20-49 years. Table 2 shows leukopaenia among control subjects, with no significant sex variation. Also, there was demonstrable significant leukocytosis ($p<0.05$) in the acutely traumatised group with mean total leucocyte count of 8194 ± 3280 cells/mm^3. The differential leukocyte count showed significant neutrophilia ($62.85 \pm 11.83\%$) and lymphopaenia ($34.82 \pm 11.38\%$).

The contribution of sex to the leukocyte profile of apparently healthy subjects and the traumatised population was found to be insignificant on Levene's t-test.

Analysis of variance test (ANOVA) was used to determine the effect of age on the observed leukocytosis of acute trauma. The p value of 0.085 indicated that age had no significant contribution to the leukocytosis of trauma

The prognostic index tested was duration of hospitalization in weeks. There was positive correlation of higher values of total WBC with length of hospital stay (Pearson correction 0.534, $P<0.05$). There was strong positive correlation between total WBC and neutrophil count (Pearson correlation = 0.295, $p<0.01$) and negatives correlation with lymphocyte count (Pearson = - 0.280)

Table1.sex distribution of the subjects with acute musculoskeletal trauma

	Frequency	Percentage	Cumulative%	Chi-squre	p-value
Male	157	76.4	70.4		
Female	66	29.6	100.0	37.135	0.001
Total	223	100%			

Table 2: Table showing the mean leukocyte profiles of apparently Healthy Nigerian compared with subjects with acute musculoskeletal trauma

Leukocyte subtype	Control group(no)	Control group (leukocyte profile)	Test group(no)	Test group(leucocyte profile)	p-value
WBC (Total) (cells/mm3)	50	4922.00±1282.264	223	8184.96±3279.623	P<0.05
Neutrophil (%)	50	53.78±12.210	223	62.85±11.827	P<0.05
Lymphocyte (%)	50	43.18±11.864	223	34.82±11.382	P<0.05
Basophil (%)	5	2.10±31.92	24	4.00 ±9.908	-
Eosinophil (%)	25	3.00±2.415	101	3.63±3.307	-
Monocyte (%)	19	2.63±1.300	59	2.97±1.742	-

DISCUSSION

The male preponderance among the subjects that sustained acute musculoskeletal trauma via road traffic accidents or clean elective orthopaedic operations (Male to female ratio of 4:1) is significantly higher than control (male;female = 3:2), ($p<0.05$). This confirms the report of earlier studies that showed higher incidence of trauma among males than females[15]. This have been attributed to the fact that the male sex at all ages is more active than their female counter-part[16]. Also Eyichukwu and Iyidobi had suggested that the greater risky behaviors of the male sex as well as their predominant roles as bread winners of the family cause the males to be more exposed to injurious circumstances[17].

Age is another demographic factor that has been documented to affect the incidence of accidental trauma in all societies. There was significant age variation in the incidence of the trauma among the test population. The results showed that trauma was much more prevalent among the young and productive age group range of 20 49years. This is the age that drives the economy and social activity of any society. Thus subjects between the ages of 20-49years accounted for about 72% of those that sustained musculoskeletal trauma. Older persons up to 70years of age are not commonly involved in road traffic accidents as majority of them are usually in retirement at home or in institutions or incapacitated by one medical condition or the other[18].

The leukocyte profiles of apparently healthy adult Nigerians (Table 2) used as control confirmed previous reports of the existence of leukopaenia in people of African descent compared with Caucasians [5]. Nwobodo *et al*, also noted a general leukopaenia among his subjects though those who had evidence of chronic infection had higher leukopaenia[36]. Earlier studies have also documented leuko-neutropania among adult Nigerians[6,] and this is consistent with African leucopaenia which has been widely, cited [5,19,20]. The true cause of African leucopaenia is yet to be resolved. Suggested causes vary from chronic infection and dietary causes to genetic factors.

The leukocyte profile of the traumatized group showed demonstrable post traumatic leukocytosis. The mean total white blood cell count was found to be 8184 ± 3280 cells/mm3. This is significantly higher than the mean for the control group (4922 ± 1282 cells/mm3) at $P<0.05$. This corroborates some of the reports by Chang (2003) who analyzed post-traumatic leukocyte count among different races[21]. This researcher postulated that only white race and severity of trauma were associated with acute increase in total white blood cell count. His conclusion that black people do not exhibit post traumatic leucocytosis could no longer subsist given the result of this present study. Thus like acute infection[3] and exercise[22] trauma also induces acute leucocytosis in Nigerians and possibly others of African origin. This finding corroborates the result of a study in Lagos in which the leukocyte response to surgical trauma in Nigeria Negros was investigated. The authors showed that the peripheral blood total leukocyte count and neutrophils (PMN) were significantly increased in one hour after major surgery and that this increase was sustained for a minimum of 7days after trauma[23]. Their conclusion was that the leukocyte and polymorphonuclear leucocyte (neutrophil) response to acute surgical trauma in the Nigeria Negro is similar to previous observations made in Caucasians[23].

The exclusion of subjects with open wounds, burns etc from the test group allows the conclusion that trauma was the underlying factor responsible for the observed leukocytosis. Early leukocytosis following trauma has been previously attributed to the presence of bacteria in blood and urinary tract infection. The investigations done towards confirming the presence of infection led to delays in the institution of appropriate treatment modalities. It has been suggested that bacteria is not the leading cause of fever and leukocytosis among those who sustain acute and severe traumatic injuries[24]. This implicates trauma as the likely cause of post traumatic leukocytosis. Similarly Golob *et al* had also shown that urinary tract infection was not the cause of majority of the observed fever and leukocytosis in the acutely traumatised[25]. They therefore concluded that emphasis need not be placed on infection as the source of fever and leukocytosis in injured subjects during the first 14days following injury since trauma also leads to inflammation and fever.

This finding is of immense physiological importance as it clearly shows the relationship between trauma, inflammation, fever and leukocytosis. Thus early damage control surgeries like debridement and fracture stabilization can be carried out as emergency[26] with a view to reducing the morbidity of unabated metabolic response to trauma and thereby improve outcome. This is even more compelling as researchers continue to point at white blood cell level as a possible indicator of severity of trauma as well as predictor of outcome. For instance, Rovalias shows that patients with severe head injury has significantly higher white blood cell counts than those with moderate or minor injury. He found a significant relationship between WBC counts and Glasgow Coma Scale scores (GCS) as well as Pupillary reaction[27]. Also, he reported very high total WBC counts among those that had unfavorable outcome. He thus concluded that WBC count was an independent predictor of outcome in severe trauma. This view is strongly supported by the correlation test in this study. In present study, duration of hospitalization, a known prognostic index was found to correlate positively with higher value of total WBC count ($p<0.05$). Patients with higher WBC counts were hospitalized for longer periods possibly because they had more severe injuries. We therefore suggest that patients with very high WBC counts could be isolated early for more aggressive modes of treatment and observation including intensive care unit (ICU) admission and early operative fixation of fractures to improve outcome and thus shorten duration of hospitalization.

There are several other advantages of using WBC count as an index of severity of injury in blunt trauma patients. The traditional parameters include Injury Security Score (ISS), Glasgow Coma Scale (GCS) and Revised Trauma Score (RTS). ISS is too complicated while GCS and RTS are subjective and observer dependent. WBC on the

other hand is easy, quick, objective and readily available and thus can be applied at least as a useful adjunct in the evaluation of severity of trauma.

There is rich evidence in the literature that trauma induced leukocytosis is mainly due to neutrophilia caused by demargination of neutrophils[28] (Santucci et al, 2008) as well as stimulation of bone marrow by cytokines elaborated acutely in trauma[29] . This is supported by the finding of a significant neutrophilia of 62.85± 11.83% among the test population in this study. Bastian *et al* clearly showed a significant acute post traumatic rise in monocyte and neutrophil levels as well as total white blood cell count among subjects who had total hip replacement arthroplasty[30]. This work confirmed that chemokine burst arising from tissue damage was responsible for the observed neutrophilia and monocytaemia. This also supports the report of Olav who noted that monocytes and macrophages are responsible for the production of the major pro-inflammatory cytokines that modulate systemic inflammatory response syndrome and subsequent organ dysfunction seen in severe trauma[26]. Thus a high absolute neutrophil count in severe trauma is associated with increased morbidity and mortality[31].

There was post-traumatic lymphopaenia among the test subjects (34.82± 11.38%). Lymphopaenia is a documented parameter following aute traumatic injury. It is seen in inverse relationship with neutrophil and has been advocated as an index of severity of trauma[15]. This inverse relationship is clearly shown by the strong negative correlation between the levels of lymphocyte and neutrophils among the test population. ($P<0.01$). Zahorec investigated the ratio of neutrophil to lymphocyte count in subjects that underwent major surgical operations and noted that the ratio in absolute and/or relative values was an easily measurable parameter which may express the severity of surgical and hence traumatic stress[32]. This is because of the consistency in their divergent and inverse values. They therefore suggested that the term NEUTROPHIL LYMPHOCYTE STRESS FACTOR (NLSF) as a ratio of neutrophil to lymphocyte counts can be of clinical use in post traumatic and other patients admitted to Intensive Care Units. This suggestion is gaining ground in literature and is supported by the result of this work. There is evidence that the near consistent poor outcome of trauma associated with extreme lymphopaenia is due to apoptosis and development of severe T-cell depletion resulting in anergy and subsequent organ failure[33].

The effect of age and sex on the leucocyte profile of the post traumatic subjects was found to be insignificant. This is contrary to documented evidence of metabolic response to trauma being more pronounced in young male adults. Waters *et al* however studied the effect of age and body composition on metabolic response to elective surgical trauma and found that serum glucose, cortisol, WBC count and c-reactive proteins were independent of age[34]. This was corroborated by the result of this study.

CONCLUSION

Adult Nigerians exhibit post-traumatic leukocytosis. Ethnic leukopaenia had no effect on the expected leukocytosis [35]. This is in contrast to suggestions that Africans do not exhibit post-traumatic leukocytosis. The post-traumatic leukocytosis was mainly from the demonstrated neutrophilia. There was demonstrable lymphopaenia among the test group. The consistent inverse relationship between the neutrophil and the lymphocyte counts (ie post traumatic neutrophilia and lymphopaenia) has been suggested to be used as an index of severity of trauma. The term NEUTROPHIL LYMPHOCYTE STRESS FACTOR (NLSF) as a ratio of neutrophil to lymphocyte counts has been advanced for use in trauma assessment and prognosis determination. This is in addition to the use of post traumatic leucocyte level, a near consistent factor in trauma as a useful index of trauma severity. Patients who had higher total WBC count stayed longer in hospital and were more likely to have sustained more severe trauma than others. This group of patients would have benefited from more aggressive treatment modalities. A multi centre double blinded study involving much more number of subjects is recommended to allow for possible clinical application of the results of this study.

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