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RESEARCH ARTICLE

EVALUATION RELEASE OF CYTOKINES (IL-6, IL-8, TNF) LEVELS IN STORED WHOLE BLOOD

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Abstract

This study was done to determine the release of cytokines (IL-6, IL-8 and TNF) in stored whole blood. Fifty (50) samples were collected from 50 subjects for the study. These comprise of 25 males and 25 females. The subjects comprise of adults aged 1 8 - 4 0 years with 29 years as the mean age. The study was conducted at GEM Research Laboratories. The IL-6, IL-8 and TNF were assayed respectively for all subjects on 0 day, 14th day and 35th day. The mean levels of IL-6, IL-8, and TNF were 10.68 pg/ml, 3.40 pg/ml, and 1.43 pg/ml respectively for 0 day, 7.56 pg/ml, 16.52 pg/ml, and 7.88 pg/ml respectively for 14th day, and 5.12 pg/ml, 124.24 pg/ml, and 59.08 pg/ml respectively for 35th day. Significant statistical difference ($p<0.05$) was observed in IL-6 and IL-8 mean levels across all groups (0/14th day, 0/35th and 14th/35th day) compared except in 0/14th where TNF mean levels showed no significant statistical difference ($p>0.05$). We recommend pre storage white cell reduction for whole blood and all red cell components.

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Introduction:-

With the aging of society, the need for life-saving emergency services is increasing. Blood transfusion is a commonly administered therapy to critically ill patients to treat conditions that result in significant morbidity or mortality that cannot otherwise be prevented or effectively managed[1]. . However, blood transfusion is an irreversible event with both potential benefits and risks for the recipient. These unwanted effects range from relatively mild to severe. Improved donor selection and antibody screening have ensured a safe blood supply, but various transfusion reactions have occurred. Acute transfusion reactions (ATR) occur within 24 hours after transfusion administration, but most occur during or within 4 hours after transfusion[2]. They can be immune and non-immune reactions. Acute immune reactions are associated with immune responses to antigens on red blood cells, white blood cells, platelets, or plasma proteins, and include acute hemolytic transfusion reactions (AHTR), febrile non-hemolytic transfusion reactions (FNHTR), and allergic anaphylactic transfusions. Immunological responses, but not associated acute lung injury (TRALI), include transfusion-related sepsis, circulatory overload, non-immunological hemolysis, hypocalcemia, and hypothermia [2].

Whole blood (WB) is still commonly used for blood transfusions in Nigeria instead of blood components. Many bioactive substances such as cytokines are released during storage[3-5]. Cytokines released from white blood cells (WBC) accumulate in the supernatant during storage of red blood cell concentrates (RCC). Leukopenia (LR) is a potential means of preventing cytokine production. The aim of this study was to estimate the levels of cytokines released during WB and RCC storage. Key cytokines such as interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha (TNF-α) play important roles in and are activated in febrile non-hemolytic transfusion reactions (FNHTR) regulates normal T cell expression and secretion. (RANTES) are primarily involved in allergic reactions[6-8].

Materials and Methods:-

Subjects and Blood Collection

Blood was collected from voluntary donors. Fifty (50) subjects aged 18-40 years were enrolled for the study. They comprise of 25 males and 25 females. The study was conducted at GEM research laboratories. Informed consents were obtained from subjects before the commencement of the study. The donors were selected according to the donor selection criteria of National Blood Transfusion Services. Blood was collected in different bags as per the requirement and stored at 2-6°C taking the day of collection as 0 day.

Ten (10) mls of blood sample were taken from each bag aseptically in laminar air flow 0, 14, 21, and 35 days. Hemogram was determined using Sysmex XP- 300- Heamatlogy Analyzer.

Estimation Of Test Parameters Cytokine Estimation

Cytokine assays were done using ELISA development kits which included a set of antibodies, standards, conjugate, and substrates. Uncoated ELISA plates were purchased separately, and ELISA for each cytokine was developed. Monoclonal antibody was coated on NUNC Maxisorpmicrowell plates overnight as per manufacturer instructions of reagents used. Each test included different dilutions of standards for the preparation of standard curves. On immobilized capture antibody detection, antibody was added followed by the addition of an enzyme and substrate producing a colour product in proportion to the concentration of cytokine. The absorbance was read using ELISA reader at 450 and 570 nm for IL-6, IL-8, and TNF-a at 405. The set of reagents used was of:

1. eBioscience (USA) for human IL-6.
2. BioLegend (San Diego, CA) for human IL-8 and TNF-a.

Statistical Analysis

Descriptive statistics which include means, standard deviation (SD), frequency and percentage were used to analyze categorical and continuous variables. Differential statistics which include chi-square were used to test association between categorical variable while independent sample t-test and one-way anova were used to compare means of continuous variables. All analysis was done using the statistical package for social sciences (SPSS) software version 20.0. P>0.05 was considered significant.

Results:-

The results were analyzed for mean, standard deviation and compared for significance between the groups using one-way analysis of variance (ANOVA) and t-test. The subjects comprise of adults aged 1 8 - 4 0 years with 29 years as the mean age. Among fifty (50) donors, thirty nine (39) were male and eleven (11) female. The haemoglobin of all donors was above 12.5 g/dl. The mean WBC count and platelet count in was 3.1 ±3.1 x 10^9 WBCs/unit and 241 ± 96 * 10^J platelets/pl. The IL-6, IL-8 and TNF were assayed for all subjects on 0 day, 14^{TH} day and 35^{TH} day.

The observed range of IL-6 on 0 day was from 1 to 30 pg/ml. A trend of decrease in IL-6 levels was observed from 0 day to 35 days. Interleukin-8 ranged between 0 to 13 pg/ml on 0 day and by the 35* day, the observed range was 27-385 pg/ml. Tumour necrosis factor ranged between 0 to 4 pg/ml on 0 day, and increased upto 156 pg/ml by the 35^{th} day of storage. The mean levels of IL-6, IL-8, and TNF were 10.68 pg/ml, 3.40. pg/ml, and 1.43 pg/ml respectively for 0 day, 7.56 pg/ml, 16.52 pg/ml, and 7.88 pg/ml respectively for 14^{th} day, and 5.12 pg/ml, 124.24 pg/ml, and 59.08 pg/ml respectively for 35^{th} day.

Interleukin-8 was in the normal range on 0 day in WB. On 7^{th} day, the level was low but increased on 14^{th} day, and there was a steady increase continuously from 14 to 35 days. On 28 days, the observed range ^as 27-402 pg/ml. Tumour necrosis factor-alpha level increased from the 14^{th} day in few samples. On 35^{th} day in one sample, the level

reached 156 pg/ml. From the work, the normal range of IL-6 was 0-30 pg/m; IL-8, 0-13 pg/ml; TNF, 0-4 pg/ml.

Table 1:- The Frequency And % Composition Of Cytokines On 0, 14th, And 35th Day.

Parameters (pg/ml)	**0 day**		**14th day**		**35th day**	
	Frequency	% composition	Frequency	% composition	Frequency	% composition
IL-6	534	68.9	378	23.7	256	2.7
IL-8	170	21.9	826	51.6	6212	65.9
TNF	71	9.2	394	24.7	2954	31.4
TOTAL	775	100	1598	100	9422	100

TABLE 2:- comparison of the mean + sd levels of il-6 on 0 day, 14™ day and 35th day.

Days	Mean + SD	t- value	P value
0/14th	10.68 + 7.61 / 7.56 ±5.83	2.301	0.024
0/3 5 th	10.68 + 7.61/5.12 + 3.81	4.619	0.000
14/3 5 th	7.56 + 5.83/5.12 + 3.81	2.477	0.015

Table 3:- Comparison Of The Mean + Sd Levels Of Il-8 On 0 Day, 14th Day And 35th Day.

Days	Mean + SD	t- value;	P value
0/14th	10.68 ± 7.61 / 7.56 ±5.83	7.968	0.000
0/3 5 th	10.68 + 7.61/5.12 + 3.81	8.337	0.000
14/3 5 th	7.56 ±5.83/5.12+ 3.81	7.396	0.000

Table 4:- Comparison Of The Mean + Sd Levels Of Tnf On 0 Day, 14th Day And 35th Day.

Days	Mean + SD	t- value	P value
0/14th	10.68 + 7.61 / 7.56 ±5.83	2.083	0.40*
0/3 5 th	10.68 + 7.61/5.12 + 3.81	9.756	0.000
14/3 5 th	7.56 + 5.83/5.12 + 3.81	7.676	0.000

Table 5:- Comparison Of The Mean + Sd Levels Of Il-6, Il- 8 And Tnf On 0 Day, 14™ Day And 35™ Day.

PARAMETERS	0 DAY Mean + SD	14TH DAY Mean + SD	35TH DAY Mean + SD	F-VALUE	P - VALUE
IL-6	10.68 ±7.61	7.56 ± 5.83	5.12 ± 3.81	10.944	0.000
IL-8	3.40 ±4.13	16.52 ± 10.89	124.24 ± 102.41	62.071	0.000
TNF	1.42 ±1.18	7.88 ±21.90	59.08 ±41.7	67.247	0.000

Table 1: Shows the frequency and percentage composition of cytokines (IL-6, IL-8, and TNF) on 0 day, 14th day, and 35th day.
Table 2: Represents the comparison of the mean + SD levels of IL-6 on 0 day, 14th day, and 35th day. There was significant statistical difference ($p<0.05$) in all the three groups (0/14th day, 0/35th and 14th/35th day).
Table 3: Represents the comparison of the mean + SD levels of IL-8 on 0 day, 14th day, and 35th day. There was significant statistical difference ($p<0.05$) in all the three groups (0/14th day, 0/35th and 14th/35th day).
Table 4: Represents the comparison of the mean + SD levels of TNF on 0 day, 14th day, and 35th day. There was significant statistical difference in TNF mean level on 0/35th day and 14/35th day ($p<0.05$) while group 0/14th day showed no statistical significance ($p>0.05$).
Table 5: Represents the comparison of the mean + SD levels of IL-6, IL-8, and TNF on 0 day, 14th day, and 35th day. There was significant statistical difference in all parameters mean level in all groups ($p<0.05$).

Discussion:-

Cytokines are not included in routine testing like other hematological parameters, so normal ranges for cytokines have not been published. The manufacturer of the kit also provided expected values depending on the samples tested. The importance of cytokines may have come to light only later, or studies may have been limited by the enormous expense of measuring cytokines. This study determined the normal ranges of IL-6, IL-8, and TNF-α in diseased whole blood from healthy donors.

Interleukin-6 showed a decreasing trend from day 0 to day 35. Similar results were reported by Jacobi et al who found IT-6 levels in whole blood to be within physiological limits[9]. A study by Nielsen et al. (1996) reported that IL-6 was below the detection limit of 3.9 pg/ml and remained low during storage[5]. These findings are therefore comparable to the present study, which concluded that IL-6 does not accumulate in whole blood stored at 4 °C.

Mean leukocyte count 3.1 ±3.1 x 10^9 WBCs/unit and platelet count 241 ± 96 x 10^3/pL in whole blood indicate that higher levels of leukocytes and platelets also contribute to increase in cytokine levels. IL-8 level increased on 14^{th} day, and there was steady increase up to 35 days. TNF-a level increased gradually after 14 days of storage. In 56% cases, the level remained 2-50 pg/ml up to 35 days of storage.

It has been estimated that a freshly collected, whole blood unit contains 10^9 leukocytes, and their concentration continues to decrease with subsequent component processing. There is a great variability in number of leucocytes in the components. The enumeration of residual leukocytes is important where traditional automated cell counters do not give accurate results at'<100 WBCs/pl. Several methods are available, but Nageotte chamber has been found to be practical and cost effective in a blood center setting whereas other techniques like flow cytometry, cytospin method etc., are expensive, cumbersome, and labour intensive. As quoted by Turner leucodepletion of blood reduces primary immunization to HLA and reduces the exposure to foreign antigens which are highly immunogenic. The rate of alloimmunization varies from 7% to 44% among recipients receiving LR products and 20% to 50% in patients transfused with non leuco-reduced products[10].

Interleukin 6 (IL-6) levels were in the normal range. There was no sample showing any value above 30 pg/ml in 35 days. This study supports the study of Sharma and Marwaha where IL-6 levels remained low at any storage time. Low levels of cytokines may have additive or synergistic effect in conjunction with other cytokines[11].

In the present study, IL-8 levels gradually increased from 0 pg/mL on day 0 to 384 pg/mL over 35 days. IL-8 has a priming effect on leukocytes, making them more sensitive to the effects of other thermogenic cytokines[12].Whole blood TNF-α levels do not increase appreciably on day 0, but increase steadily from day 14 onwards.

Conclusion:-

Stored whole blood may release leukocyte- and platelet-derived cytokines in a time-dependent manner and may be associated with many transfusion reactions. Comparative levels of cytokines show a time-dependent increase in IL-8 and TNF-α in whole blood. Using a buffy coat-depleted blood component can prevent many side effects from white blood cells and cytokines.For whole blood and all red blood cell components, it is recommended to deplete white blood cells before storage.

Conflicts Of Interests

The authours that there is no conflict of interest.

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