ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTEMENT OF MEDICAL LABORATORY SCIENCES

Selected Hematological and biochemical profiles of burn patients visiting Yekatit 12 Hospital and AaBET Hospital, Addis Ababa, Ethiopia

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Abbreviations

AAU	Addis Ababa University
ALT	Alanine Amino transaminase
AST	Aspartate Amino transaminase
CBC	Complete Blood Count
DRERC	Departmental Research and Ethics Review Committee
EDTA	Ethylene diamine tetra-acetic acid
HCT	Hematocrit
HGB	Hemoglobin
LFT	Liver Functional Test
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MPV	Mean Platelet volume
PDW	Platelet distribution width
PLT	Platelet
QC	Quality Control
RBC	Red Blood Cell
RDW	Red cell distribution width
RFT	Renal Functional Test
K^+	Potassium
Na^+	Sodium
SOP	Standard Operating Procedure
SST	Serum Separator Tube
TBSA	Total Body Surface Area
WHO	World Health Organization

Abstract

Background: Burn injuries are classified as one of the most devastating of all injuries and major global public health crisis. It is also one of the injuries which have pronounced effects on changing body biological parameters. However, little is known with regard to strategic management of burn patients by using an expected alteration of hematological and biochemical parameters in Ethiopia.

Objectives: To determine selected Hematological and biochemical profile of burn patients at Yekatit 12 medical hospital and AaBET Hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Methods: A prospective cross-sectional study was conducted among 72 burn patients and 71 control groups from October, 2020 to July, 2021 at Yekatit 12 and AaBET medical hospitals, Addis Ababa, Ethiopia. Complete blood Count and biochemical assays were performed using Beckman coulter and Roche Diagnostic Cobas C311 automated analyzers respectively. Data was entered, analyzed and interpreted by using SPSS version 24. Association of selected Hematological and biochemical profile were done with the severity of burn. Statistical significance was defined as p value < 0.05 with 95% confidence interval.

Results: Of the total 72 burn patients, 37(51.4%) were females and 32(44.4%) were children aged from 1 to 15 years old. Of the total 71 controls, males were 36(50.7%) and 39(54.9%) were adults >30 years old. Both burn patients and control groups resided in Urban areas 42(58.3% and 36(57.7%) respectively. Most injuries were Second degree burn 46(63.9%) caused mainly by Scald 31(43.1%) with 10-20% Total Body Surface Area (TBSA %) affected in the majority of cases (29(40.3%)). The majority of the Scald occurred in young children aged 1-15 years old 17(53.1%) Blood WBC count was initially raised and gradually decreased. HGB and HCT level was low during admission as compared to control group and increased on 1^{st} and 2^{nd} weeks. RBC count was elevated during admission comparing with control and then decreased. Serum creatinine and urea levels decreased on admission, 1^{st} and 3^{rd} weeks but showed increment on 2^{nd} week. Serum AST levels increased on admission and 3^{rd} week. But it decreased on 1^{st} and 2^{nd} weeks. With regard to serum ALT levels, there was a significant increment ($p<0.05$) on admission day, non-significant ($p>0.05$) increment on 1^{st} week and 3^{rd} week and there was a decrease on 2^{nd} week. In case of serum Na^{+} electrolyte concentration, there was decrement on admission and 1^{st} week and increased on 2^{nd} and 3^{rd} weeks. With regard to serum K^{+} concentration, it decreased on admission and on 3^{rd} week, but there was an elevation on 1^{st} and 2^{nd} weeks. Severity of burn high (TBSA %) had a direct relationship with WBC mean change but it was inversely related to platelet mean change. There was slightly high change of biochemical profile with high TBSA value.

Conclusion: Various selected hematological and biochemical changes have been observed in burn patients. Thus this finding can be helpful to the health care team to make careful monitoring of these hematological and biochemical parameters which can help improve the health of burn patients, reduce mortality rate and improve survival.

Key words: Hematological parameters, Biochemical parameters, Serum electrolytes, severe burn.

1. INTRODUCTION

1.1 Background

Burn injury is an injury which is the most destructive of all injuries and a major public health problem in the world (1). According to the World Health Organization (WHO) report in 2017, burn results in more than 7.1 million injuries, the loss of almost 18 million disability adjusted life years and more than 250,000 deaths occur each year worldwide. The majority of this problem (90%) occurs in the countries of Africa (2).

The degree of damage to tissue depends on the duration of contact with the burn agent, the nature of agent that causes the burn and on the severity of heat on contact site (3). According to the depth of injuries, burn is classified as First degree:- in which the superficial skin is injured (epidermis); Superficial Second degree:- by which in addition to the superficial skin damage the superficial dermis is also damaged; Deep Second degree:- in which the epidermis and deep to dermis damage occurs; Third degree burn:- in this burn full thickness injury through the epidermis, dermis into subcutaneous fat damage occurs and the Fourth burn degree:- starting from the superficial skin to underlying muscle and bone (1). Mostly the known causes of burn are heat, electricity, hot liquid (scalds), chemicals or radiations.

There are various health problems that occur because of burn injury that actually depend on its severity and the body surface it gets in contact. Burn injury is associated with anatomical, physiological, biochemical and immunological alterations. Infection is a problem which occurs on burned area during healing or as soon as accident happens. Burn patients are at risk of developing infection as a result of the nature of the burn itself, the effects of burn (it is immunocompromising), hospital stay and intensive diagnostic and therapeutic procedure. Sepsis of burn wounds is serious problem that can be one cause for death (4).

The incidence of infection because of burn is higher than other forms of trauma and the reasons are the damage of skin barrier and disruption in cellular and humoral responses (3). In addition to infection, there are complex pattern of response caused by severe burn which is an inflammatory response is triggered to promote the healing process. However, in severe burns, this inflammatory process can be extensive and become uncontrolled, leading to an augmented

inflammation that does not induce healing but rather causes a generalized catabolic state and delayed healing. This response is almost unique to burns and is referred to as the hypermetabolic response; it is associated with catabolism, increased incidence of organ failure, infections and even death (5).

As it is reported by Cakir.B.et al Local and systematic inflammatory response is extremely complex, resulting in both local burn tissue damage and deleterious systemic effects on all other organ systems distant from the burn area itself. Local inflammatory response occurs immediately after injury but systematic inflammatory response will take time to respond usually 5 to 7 days. Thermal injury induces the production of burn toxins and oxygen radicals and finally leads to peroxidation which is responsible for the further damage of organ in burn injuries. The injured tissue initiates an inflammation-induced hyper dynamic, hypermetabolic state that can lead to severe progressive distant organ failure (6). The pathological response of injured body depends on the severity of the injury and how much time is taken for expected treatments, as time elapsed for treatment, therefore the local response will be followed by systematic response which will impose pathological changes on respiratory and circulatory system which is complex (1).

Thermal injury has effects on the alteration of biochemical and hematological parameters. The first consequence of thermal injury is burn shock which is a hypovolemic shock resulting from the disturbances in membrane permeability accompanied by oedema, exudation and evaporation. These will be followed by plasma loss, haemoconcentration, increased blood viscosity and all haemodynamic consequences (3)(7). Systematic enzymatic alteration will occur following the burn from the metabolical and biochemical changes:- increased free radical activity, blood levels of ascorbic acid, uric acid and elevation of the activity of some serum enzymes. Changes in the serum transaminases (AST and ALT) and alkaline phosphatase (ALP), creatinine kinase, lactate dehydrogenase-glutamyl trans peptidase have been reported in burn patients (8).

1.2 Statement of the problem

Burn injuries are among the most destructive of all injuries and a major global public health problem. As WHO reported, annually the death rate of burn patients is more than 250,000 and almost two third of this occur in Africa. The non-fatal burn injury is the leading cause for morbidity including disfigurements, disability and prolonged hospitalization (2).

As the 2004 WHO report indicated nearly 11 million people worldwide were in need of medical attention because of burn injury. As one study done in Addis Ababa indicated burn injury is an important health problem in Ethiopia and in this study 11.6% was the mortality rate of burn patients. Therefore, burn injury is a major public health problem in the country which needs more study for patient's management (9).

Burn is also a profound and devastating injury which has effects on the alterations of anatomical, physiological, biochemical and immunological body health setup. As body gets burned there are local and systematic response which needs management, the pathological changes that occur on respiratory and circulatory systems are complex and it needs understanding its progress and therapeutic management (10).

Burn injury change body's physiological parameters which disturb the cellular hemostasis, it will increase or decrease hematological and biochemical parameters that contribute to hyper metabolic state which arises mainly due to increase of adrenaline release, loss of fluid and electrolyte, hemolysis and sepsis. As it is known the levels of these substances are very important in supporting the health care team to know how the body is responding to the different therapies that are being provided and this will help the medical staff for proper management with less morbidity and mortality (11).

In Ethiopia, there is no research conducted so far on what do changes in parameters of hematology and biochemical Sims like after burn injury. Therefore, this study will provide selected Hematological and biochemical profile of burn patients so as to provide timely and safely treatment for burn patients.

1.3 Significance of the study

As burn injury has sequential and scientific managements of the problem, medical staff needs medical characteristics of the patients which are used as basements for delivering treatments, therefore having information about what really changed in hematology and biochemical parameters according to our segments will help physicians for management strategy and outcome of treatment. It can also be used as base line data for further study in our settings.

2. Literature Review

2.1 Hematological profile of Burn patients

As burn injury dysregulates and disturbs the body's homeostasis it has effects on hematological parameters. There are various studies done on how burn injury alters the hematological profile. A retrospective study done in United States (2017) in a total of 191 patients found that following a severe burn injury, significant hematologic changes occur that are reflected in complete blood count (CBC) measurements. Hemoglobin and hematocrit decreased over the first week, this decrease is because of red blood cells loss. WBC counts initially elevated then decreased over the first 4 days. As they studied PLT also decreased over the first 4 days (1**1**).

A prospective observational cohort study done in UK in 2017 showed that sepsis is the major cause for mortality. The circulating Platelet is normal on first day of injury and then it significantly decreased on day 3. It indicated neutrophil is significantly increased on day of post injury. Red cell fragmentation formed because of direct damage to circulating red blood cell of thermal injury this cause overestimation of platelet counts by impedance analyzers **(12)**.

A cross-sectional study done in 50 patients of thermal burn admitted in the Burn ward of M.B. Govt. Hospital, R.N.T Medical College, Udaipur, India during 2014-16, showed that initially hemoglobin and hematocrit levels rise and progressively get decreased. The total leucocyte count initially increased. It also point as corrected hematological disorder led to improvements on morbidity and less mortality. And they also reported that thermal burn occurs more in male than in female **(13).**

A retrospective cross sectional study done in Korea in 2011 on sample number of 265 burned patients revealed that the pattern of hematological parameters is dependent on burn size and time after burn injury, leukocyte, hemoglobin and platelet count began to increase immediately after burn injury, reaching peak within 12 hours after injury and then decreased. As this study documented leukocyte count is recorded as lowest in 3 to 4 days and began to increase. In case of hemoglobin it continuously decreased and remained at the level of anemia from day 4 to 14. Platelet count is recorded lowest in 3 to 4 days and then come to be continuously increased until

day 14. Finally this study concluded, as the burn size get wider change in leukocyte, hemoglobin and platelet will be high (1**4**).

The prospective study done in Egypt (1996) on 30 patients revealed that the concentration of hemoglobin showed significantly increased levels immediately after burn and gradually decreased below control level by day 6 post burn. The same pattern was noticed in hematocrit also. There is also high level of leukocyte. On contrary they reported platelet as it significantly decreased. They conducted their study by recording daily burned patient status and reported that the severity of burn related to degree of anemia (10).

2.2 Biochemical profile of Burn patients

As there are changes in hematological parameters there are also pronounced changes that occur in biochemical profile which in turn have serious damage. There are many studies done on this issue. Facility based cross sectional study done on sample size of 30 patients in India in 2015 presented some facts on the biochemical changes, the formation of edemas which have serious effect on the cell. It may lead to damage. As this study showed there are releasements of liver enzymes (such as aspartate aminotransferase (AST) and alanine amino transferase (ALT)) which can be seen as an indicatory of hepatocyte injury. Cellular injury or changes in cell membrane permeability, leak these enzymes into circulation. Low Na and high K+ occurred because of increased vascular permeability and cell edema. In this way they found highly significant increments of 23 times elevation in AST, 21 times in ALT, 4 times increase in ALP and 1.7 times decrease in sodium and 1.4 times increase in potassium levels (1**5).**

A case control study done in India during a period of January, 2014 to September, 2015 on 30 female burn patients and 30 healthy female as control showed that AST and ALT levels were significantly raised because of an increase in edema formation which led to liver cell damage, with release of hepatic enzymes. In this study, they showed burn injury could have a direct impact on hepatic organ that can be indicated by hepatocyte enzymes elevation (**15**).

The case control hospital based study conducted on 60 patients in Egypt in 2018 showed that, there were significant increments in blood urea and serum creatinine levels in burn patients in

comparison to control. The serum Na+ and K+ levels decreased significantly during the 3rd and 5th days post burn (**16**).

A case-control longitudinal study involving a total of 106 subjects made up of 53 cases (thermally burnt patients) recruited between the period from September, 2015 to April, 2016 at the burns center of the Korle Bu Teaching Hospital(Ghana) showed Serum sodium and magnesium levels significantly low on ≤ 2nd, 7th and 14th days post thermal burns, with ≤ 2 days recording the lowest serum sodium level in all age group. whereas serum potassium levels were significantly high on ≤ 2nd, 7th and 14th days post thermal burns. Also they showed serum urea and serum creatinine was significantly low specially less than 2nd , 7th and 14th days (1**7**).

3. Objectives

3.1 General objective

To determine selected Hematological and biochemical profiles of burn patients visiting Yekatit 12 Hospital and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

3.2 Specific Objectives

- To determine the magnitude of selected Hematological parameters in burn patients
- To measure selected parameters of biochemical in burn patients
- To measure the correlation of changes in selected body parameters and severity of burn injury

4. Materials and Methods

4.1 Study area (site)

The study was conducted in Yekatit 12 hospital and AaBET Hospital. Yektatit 12 hospital is one of the hospitals under Addis Ababa city administration health bureau that has been giving routine health services for Addis Ababa and other referral cases from different regional states of Ethiopia. The hospital provides services for a population of approximately 4 million people. It has 9 departments and 6 units and has 265 beds. It has been the main referral hospital for treatment of severe burns for many years. The burn unit has 19 beds of which 7 of them are reserved for pediatric burn victims.

Addis Ababa Burn, Emergency and Trauma Hospital (AaBET Hospital) is an affiliate of St. Paul's Hospital Millennium Medical College and is inclusive of four major departments; Emergency Medicine and Critical Care, Plastic Reconstructive and Hand Surgery, Orthopedics and Traumatology, and Neurosurgery. AaBET Hospital is progressive and successfully managed upper-level teaching hospital with 190 and 190 bed-ward departments as well as centers for traumatology, physiotherapy, and spinal disorders. The facility is recognized as one of the largest government-based trauma and emergency center in the country.

4.2 Study design and period

A prospective cross sectional study was used to asses selected Hematological and biochemical parameters, within period of October, 2020 to July 2021.

4.3 Population

4.3.1Source Population

The source population was all burn patients attending Yekatit 12 Hospital and Addis Ababa Burn, Emergency & Trauma Hospital (AaBET Hospital) during the study period.

4.3.2 Study Population

The study populations were all burn patients attending Yekatit 12 Hospital and AaBET hospital that fulfilled the inclusion criteria. In addition, apparently healthy patients' guardians that volunteered were used as a control group.

4.4. Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Willingness of patients and control groups for giving necessary information

4.4.2 Exclusion Criteria

- Cardiac, hepatic, malignant and renal patients (from participated burn patients)

4.5 Study Variables

4.5.1 Dependent Variable

- Hematological parameters
- Biochemical profile

4.5.2 Independent Variable

- Socio-demographic characteristics (age, sex)
- Degree of burn injury
- Types of burn injury
- Total body surface area burnt (TBSA %)
- Time duration between injury and hospital admission
- Effect of Medication used

4.6 Sample size and Sampling Method

4.6.1 Sample size calculation.

All burn patients visiting burn OPD and burn unit (BU) within the data collection period were included in the study. Equal number of control groups (from apparently healthy patients'

guardians that volunteer) was also included in the study. Convenient sampling technique was implemented to select both the study and control groups.

4.7 Data collection procedure

4.7.1 Socio-demographic data collection

A standardized Data Extraction Form (Annex IX. A) Was used to extract socio-demographic characteristics and clinical information of the study participants from Yekatit 12 and AaBET Hospital registration logbook by the principal investigator. After signing an informed consent, relevant information concerning socio-demographic characteristics of the control groups were also collected using a standardized data collection form (Annex IX. B).

4.7.2 Laboratory sample collection and preparation

An 8mL of venous blood was collected by following the Standard Operating Procedure for performing venipuncture (Annex XI A) by trained sample collectors from each consenting burn patients and control groups into two groups of labeled tubes; the first tubes contained EDTA as anti-coagulants to prevent clotting of blood to be used for hematological studies. The second group tubes were without anti-coagulant as plain tubes, for blood used for preparing sera for subsequent biochemical tests.

4.8 Laboratory Investigation

4.8.1 Hematological Analysis

Complete blood count (CBC) was performed on burn patients blood at time of admission (Burn A), then repeated at first week post burn (Burn B), on 2nd week from admission (Burn C), and 3rd week from admission (Burn D), using Beckman coulter Hematology analyzer. CBC was also performed on control groups as well.

Principle

Hematological analysis is concerned with the analysis of the cellular component of blood including tests that are used for evaluation of erythrocytes, leucocytes and platelets. Blood sample for complete blood count must be collected on EDTA as anticoagulant.

In this study CBC was performed using Beckman coulter Hematology analyzer (Annex XI B). This analyzer uses Electrical impedance method to count blood cells quickly by measuring the changes in electrical conductance as cells suspended in a conductive fluid passed through a small

orifice. The Coulter principle states that particles (cells) pulled through an orifice, concurrent with an electric current, produce a change in impedance that is proportional to the volume of the particle traversing the orifice. This pulse in impendance originates from the displacement of electrolyte caused by the particle. The Coulter principle was named for its inventor, Wallace H. Coulter (18).

4.8.2 Biochemical Analysis

Selected biochemical analysis was performed on burn patient's serum at time of admission (Burn A), then repeated at first week post burn (Burn B), on 2nd week from admission (Burn C), and 3rd week (Burn D). Selected biochemical analysis was also performed on control group as well. Then a statistical comparison between the individual data of the selected biochemical changes on the 72 burn patients and 71 control groups was made.

Spectrophotometric measurements of ALT, AST, ALP, Serum electrolytes (Na+ and K+), blood urea and serum creatinine was performed using Cobas C311 (Roche diagnostics, Germany) (Annex XI C).

The Roche Diagnostic Cobas C311 analyzer is automated, software-controlled analyzer for clinical chemistry analysis. It is designed for both quantitative and qualitative *in vitro* determinations using a large variety of tests for analysis. The Cobas C311 analyzer performs photometric assays and ion selective electrode measurements and uses serum/plasma (19).

4.9 Data Management and Data quality assurance

4.9.1 Pre-Analytical

- The quality of data extracted for socio-demographic and clinical information of the study participants were ensured by carefully extracting data from the registration log book using standardized data collection form. The quality of socio-demographic data collected for the control group was also ensured by carefully filling out a separate standardized data collection form. In both cases, the collected data was checked for completeness and monitored throughout the data collection as well as data entry and analysis by the

principal investigator. All blood samples collected were labeled properly. Blood sample quality was ensured by collecting and processing according to the Standard Operating Procedures (SOPs) (Annex XI A).

4.9.2 Analytical

- All reagents and controls used for testing was stored and handled appropriately as per the manufacturer's instruction.
- The machines used both for Hematological and Biochemical analyses were checked for their proper function before use.
- All samples were analyzed after the daily control running.
- Appropriate reagents were used for each analysis.

4.9.3 Post analytical

Every day, the collected data were checked for completeness and accuracy by the principal investigator. During the entry of data it was cross checked to assure the right data had been entered correctly.

4.10 Data processing and Statistical Analysis

The collected data were checked, cleaned, and analyzed using SPSS version 24. Descriptive statistics were performed and presented using frequency, percentages, mean (SD), based on the scales of the variables. Student-*t*-test for continuous variables was used to assess the relationships between the variables of interest. Percentages, tables and graphs were used for depiction of the results. P-value of less than 0.05 was used to declare the statistical significance.

4.11 Ethical considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, and Department of Laboratory Sciences. Permission was obtained from Yekatit 12 and AaBET hospital managements based on the support letter from the Department. The study was carried out after obtaining informed consent and samples was coded and

confidentiality of patient data was maintained throughout the study by locking hard copies and password protection of electronic data.

4.12 Result dissemination

This study could serve as a reference material to physicians or any health professionals, researchers, experts and policy makers for intervention. To reach these bodies the finalized paper was submitted to Addis Ababa University, College of Health Sciences, Department of Laboratory Sciences. Additional effort will also be made to present on conferences to reach the medical/scientific community and publish the article on reputable Journals.

5. Results

5.1 Socio-demographic characteristics, degree, types and severity of burn in burn patients

In this study a total of 72 burn patients and 71 apparently healthy control groups were included. Females accounted for 37(51.4%) of the burn patients while 35(49.3%) of the control group. The majority of study population were children (1-15 years old) 32 (44.4%), followed by adults (>30 years old) 23 (31.9%) and lastly young people (15-30 years old) 17(23.6%). Whereas in the control group, the majority were adults 39(54.9%) followed by young people 28(39.4%) and children 4(5.6%). Both burn patients and control groups resided in Urban areas 42(58.3% and 36(57.7%) respectively (Table 1).

With regard to the degree of patients burn, 46 (63.9%) of them had Second degree burn and 26 (36.1%) of them had Third degree of burn. Most of the burn injuries were caused by Scald 31 (43.1%) followed by Flame 27(37.5%) and least cases were caused by Electrical 14 (19.4%). many number of the patients 29(40.3%) was in (10-20) % (TBSA %) category (Table 2).

In our study, Scald burn occurred in high frequency in both males 16(45.7%) and females (ratio M:F 1.06 :1). With respect to age distribution, scald occurred twice as high in 1-15 year age category. In flame burn type, females were affected four times higher than males and it was higher in number in (1-15 year) age category. In case of electrical burn, females were totally absent whereas males were affected 14 (40.0%). High number of electrical case was recorded in (>30 year) age category (Table 3).

Table 1. Socio-demographic characteristics of burn patients and control groups at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Variables			Frequency	Percentage (%)
Sex	**Patients**	**Male**	**35**	**48.6**
		Female	**37**	**51.4**
	Control	**Male**	**36**	**50.7**
		Female	**35**	**49.3**
Age	**Patients**	**1-15**	**32**	**44.4**
		15-30	**17**	**23.6**
		>30	**23**	**31.9**
	Control	**1-15**	**4**	**5.6**
		15-30	**28**	**39.4**
		>30	**39**	**54.9**
Residence	**Patients**	**Urban**	**42**	**58.3**
		Rural	**30**	**41.7**
	Control	**Urban**	**36**	**57.7**
		Rural	**35**	**42.3**

Table 2. Burn patient's degree of burn, burn types and Total Body Surface Area burnt (TBSA%) at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Variables		Frequency	Percentage (%)
Degree of Burn	**Second Degree**	**46**	**63.9**
	Third Degree	**26**	**36.1**
Type of Burn	**Scald**	**31**	**43.1**
	Flame	**27**	**37.5**
	Electrical	**14**	**19.4**
TBSA (%)	**<10**	**19**	**26.4**
	10-20	**29**	**40.3**
	>20	**24**	**33.3**

Table 3. Sex and age distribution of Type of burn in burn patient's at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Type of Burn	Scald	Flame	Electricity
Sex n (%)			
Male **Female**	**16(45.7%)** **15(40.5%)**	**5 (14.3 %)** **22(59.5 %)**	**14 (40.0%)** **Null**
M:F ratio	**1.06 :1**	**1:4.4**	**1:0**
Age, years, n (%)			
1-15 **15-30** **>30**	**17(53.1%)** **8 (47.1%)** **6 (26.1%)**	**12 (37.5%)** **4 (23.5 %)** **11 (47.8%)**	**3 (9.4%)** **5 (29.4%)** **6 (26.1%)**

5.2 Hematological profile

The mean white blood cell WBC ($10^3/\mu l$) count increased significantly in burn patients when compared with the control group (P1=0.000) with 95%Confidence interval (-5.56673,-3.14629) but continued decreasing in the first week (P2=0.271) 95%CI (-3.10703, 0.88073), second week (P3= 0.475) 95%CI (-1.60541, 3.40039) and third week in a non-significant manner (P4= 0.019) 95%CI (0.53263, 5.62433) (Table 4). (Figure 1) (Key = Student-*t* test where P<0.05= significant value, P1 comparison between control and baseline, P2 comparison between baseline and first week, P3 comparison between first week and second week, P4 comparison between second week and third week of analysis).

RBC (10^3/ µl) count increased non-significantly during admission day (P1= 0.867) 95% Confidence Interval of the Difference (-0.38862, 0.32797) in burn patients when compared to the control group; on first week it non-significantly increased (P2=0.584) 95%CI(-0.62986, 0.35693) compared with the baseline and decreased gradually after the second week of diagnosis non-significantly ((P3= 0.881) 95%CI(-.62061, 0.72125) and (P4= 0.173) 95%CI(-1.10675, 0.20675)) (Table 4).

Blood HGB (g/dl) concentration of burn patients was low significantly (P1=0.012) 95%CI (-2.05736, -0.25335) during admission day when compared with apparently healthy control groups; while in the first week, and in the second week it increased non-significantly when we compared with baseline and first week respectively (P2=0.756) 95%CI (-1.58070, 1.15126), P3= 0.653) 95%CI (-1.58070,1.15126)), and then decreased on third week (P4=0.190) 95%CI(-.70276, 3.42473) when compared with second week result (Table 4) (Figure 2).

Hematocrit (HCT) was non- significantly decreased on admission day (P1=0.761) 95%CI (3.662, 2.686), 1st and 3rd weeks when compared to control, baseline case and second week respectively. A non-significant increment (P3 =0.709) 95%CI (-5.811, 3.980) was recorded only in second week when compared to first week analysis (Table 4) (Figure 2).

Regarding blood platelet (10^3/µl) count, it increased significantly (P1=0.000) 95%CI (-190.71125, -100.67235) in burn patients during admission day when compared with the control group and decreased non-significantly on first week when compared to baseline (P2= 0.153) 95%CI (-105.14402, 16.75869). Even though it was higher when compared with the control group, it decreased in a non-significant manner in the second week (P3= 0.157) 95%CI (-128.28085, 21.16335) and third week (P4= 0.650) 95%CI (-128.23194, 80.98051) (Table 4) (Figure 3).

Table 4. Comparison of WBC,RBC, HGB, HCT and PLT count between control and case (baseline), between case (baseline) and first week, between first week and second week, between second week and third week of analysis of burn patients at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Complete blood count parameters	Baseline case N=72	Control N=71	First week case N=32	Second week case N= 26	Third week case N= 14	P1 - value	P2 - value	P3 - value	P4 - value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
WBC(10^3/µl)	12.21±4.51	7.85±2.45	11.10±4.91	10.20±3.95	7.12±2.38	0.000	0.164	0.475	0.019
RBC(10^3/µl	4.70±1.86	4.57±1.28	4.74±1.58	4.72±1.50	4.08±0.53	0.867	0.584	0.881	0.173
HGB(g/dl)	12.51±3.02	13.66±2.38	12.72±3.68	13.15±3.46	11.79±2.13	0.012	0.756	0.653	0.190
HCT (%)	37.40±8.81	37.89±8.27	35.80±9.81	36.72±8.51	35.63±5.67	0.761	0.411	0.709	0.681
PLT(10^3/µl)	413.63±170.51	247.78±126.95	349.28±146.69	388.46±147.02	379.21±191.94	0.000	0.153	0.157	0.650

Key = **WBCs** (white blood cells), **PLTs** (platelets), **HGB** (hemoglobin), **RBCs** (red blood cells), **HCT** (Hematocrit value), Student-t test where **P**<0.05= Significant value, **P1**=comparison between control and Baseline data, **P2**=comparison between 1st week case and baseline data, **P3**=comparison between 2nd week case and first week case, **P4**= comparison between 3rd week case and 2nd week case.

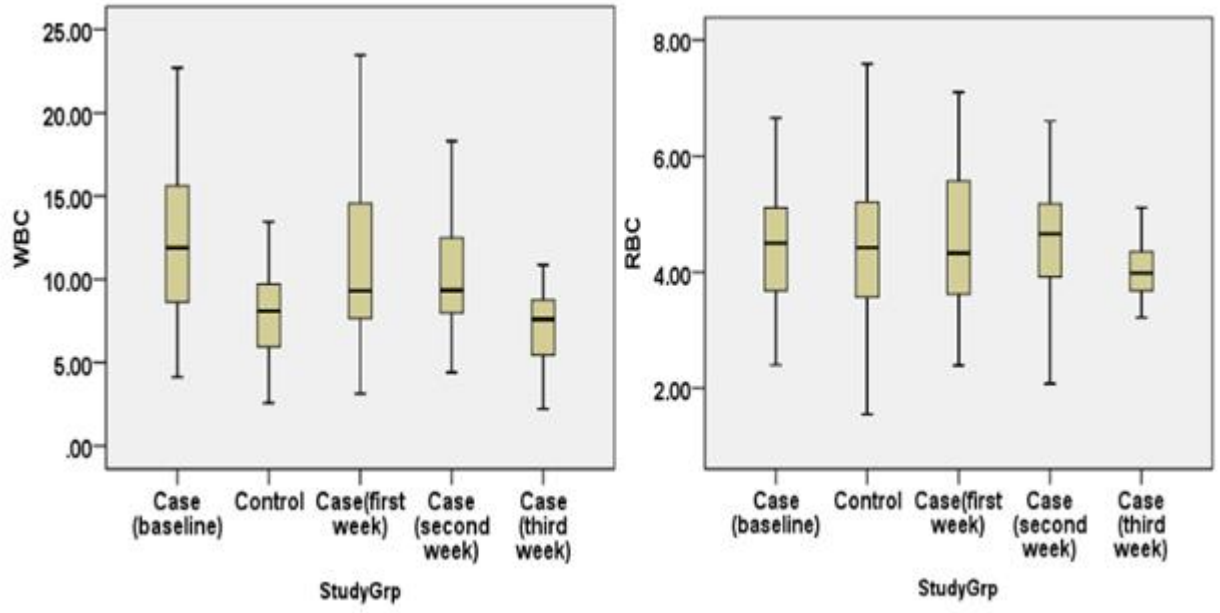

Figure 1. WBCs and RBC mean level (x10^3/µl) in burn patients in comparison to control and each successive weekly data.

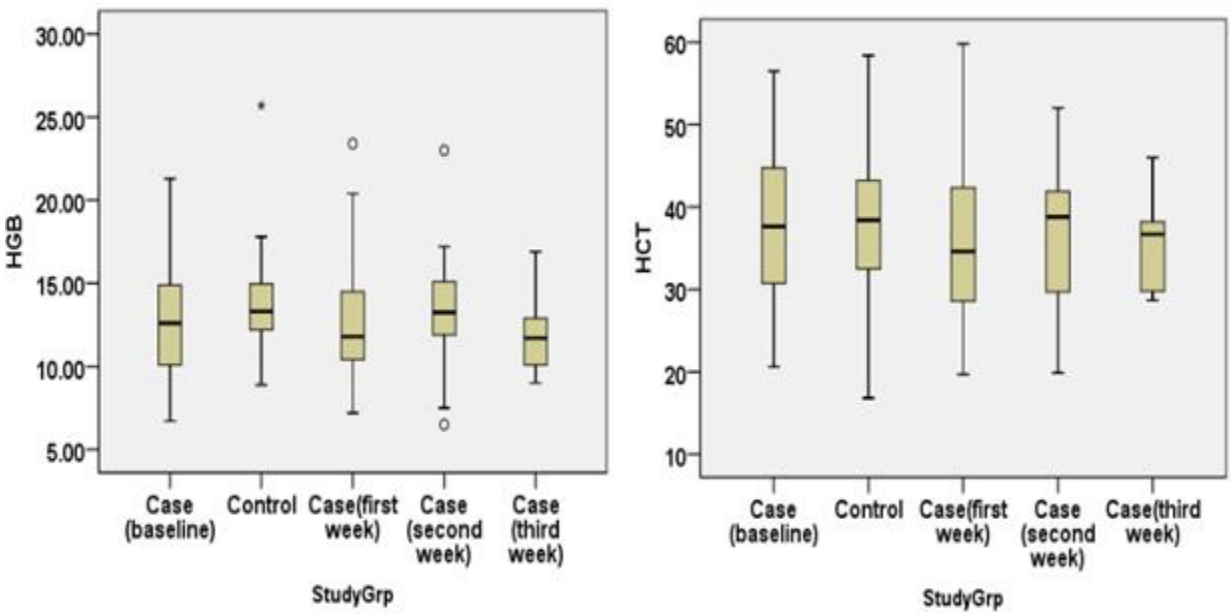

Figure 2. HGB (g/dl) and HCT (%) in burn patients in comparison to control and each successive weekly data.

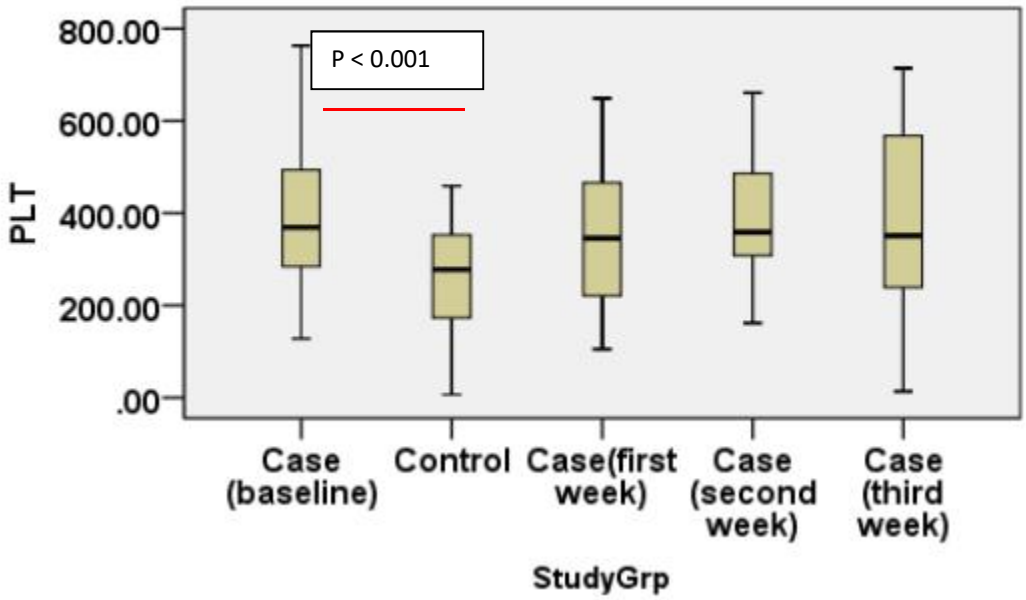

Figure 3. PLT(x10^3/µl) in burn patients in comparisons to control and each successive weekly data.

5.3Selected Biochemical profile

In case of biochemical profile of serum creatinine (mg/dl) level of burn patients, it decreased non-significantly on admission day when compared to control group (P1=0.957) 95%CI (-0.29765, 0.28171). It also decreased non-significantly on first week (P2=0.446) 95%CI (-0.26542, 0.59848) and third week (P4=0.654) 95%CI (-0.19729, 0.31070) compared with the base line data and with second week respectively. Whereas in second week, it increased non-significantly when compared to first week (P3= 0.364) 95%CI (-0.09659, 0.25928) (Table 5) (Figure 4).

Serum urea (mg/dl) level significantly decreased (P1=0.000) 95%CI (-15.76226, -6.74255) on admission day when compared to the control group, and it also non-significantly (P2=0.495) 95%CI(-3.64011, 7.48580) decreased on first week compared to baseline data, but in the second week it non-significantly (P3=0.356) 95%CI(-3.43557, 9.38855) increased when compared to first week and on the third week it non-significantly (P4=0.530) 95%CI(-6.19901, 11.84253) decreased when compared to second week (Table 5) (Figure 4).

Serum AST level significantly (P1=0.007) 95%CI (9.197065, 5.62453) increased on admission day when compared to the control group; while on first week, it non-significantly (P2=0.819) 95%CI(-31.95328, 40.31092) decreased compared with admission days data; it also decreased significantly (P3=0.039) 95%CI(-39.40950, -1.08195) on second week when compared to first week data and on the third week it non-significantly (P4=0.156) 95%CI(-31.74965, 5.28767) increased compared with second week data (Table 5) (Figure 5).

In case of serum ALT, it significantly (P1=0.039) 95%CI (0.83554, 0.31725) increased on admission day when compared with the control group, and it also increased non-significantly (P2=0.823) 95%CI (-26.05380, 20.74825) on the first week compared with baseline data. On the second week, it non-significantly (P3=0.120) 95%CI (-29.20187, 3.44033) decreased when compared to first week analysis, and lastly on the third week it non-significantly (P4=0.992) 95%CI (-10.69201, 10.58047) increased by small number when compared to second week (Table 5) (Figure 5).

With regarding to serum Na^+ Electrolyte concentration, on the first day of admission it significantly (P1= 0.000) 95%CI (-16.91675, -7.89927) decreased compared with the control group, and it also continued to decrease non-significantly (P2=0.677) 95%CI (-5.20565, 7.97926) on first week when compared to admission day. On the second week, it got higher non-significantly (P3=0.505) 95%CI (-3.48550, 6.99608) compared with first week and the same trend was recorded on the third week (P4= 0.398) 95%CI (-9.63049, 3.91071) when compared to second week (Table 5) (Figure 6).

In case of serum K^+ concentration, on admission day it non-significantly (P1=0.111) 95%CI (-1.11898, 0.11647) decreased when compared to the control group, and in first week of diagnosis this electrolyte non-significantly (P2=0.395) 95%CI (-1.10595, 0.43997) increased when compared with base line data. In the second week of analysis, it non-significantly (P3=0.144) 95%CI (-.37466, 2.51244) increased compared with first week. But in the third week it non-significantly (P4=0.089) 95%CI (-0.24335, 3.25918) decreased when compared to second week (Table 5) (Figure 6).

Table 5. Comparison of Serum Creatinine, Urea, AST , ALT, Na^+ and K^+ level between control and case (baseline), between case (baseline) and first week, between first week and second week, between second week and third week of analysis of burn patients at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

Selected biochemical parameters	Baseline case N=72	Control N=71	First week case N=32	Second week case N= 26	Third week case N= 14	P1 - value	P2 - value	P3 - value	P4 - value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Creatinine(mg/dl)	0.66±1.22	0.67±0.19	0.50±0.21	0.58±0.44	0.52±0.21	0.957	0.446	0.364	0.654
Urea(mg/dl)	22.88±14.26	34.13±12.97	20.95±10.35	23.93±14.01	21.11±12.27	0.000	0.495	0.356	0.530
AST(IU/L)	60.95±98.38	28.53±10.48	56.77±44.91	36.52±20.94	49.75±37.18	0.007	0.819	0.039	0.156
ALT(IU/L)	37.74±61.62	22.17±12.33	40.40±38.07	27.51±18.31	27.57±9.43	0.039	0.823	0.120	0.992
Na^+(Mmol/l)	130.69±17.68	143.10±7.56	129.30±9.41	131.06± 10.48	133.92±9.27	0.000	0.677	0.505	0.398
K^+(Mmol/l)	4.67±1.54	5.17±2.14	5.00±2.36	6.07±3.12	4.56±1.05	0.111	0.395	0.144	0.089

Key= **ALT** (alanine transaminase); **AST** (aspartate transaminase), **Na^+** (sodium) and **K^+** (potassium) Student-t test where P<0.05= Significant value, **P1**=comparison between control and baseline data, **P2**=comparison between first week case and baseline data, **P3**=comparison between second week case and first week case, **P4**= comparison between third week case and second week case.

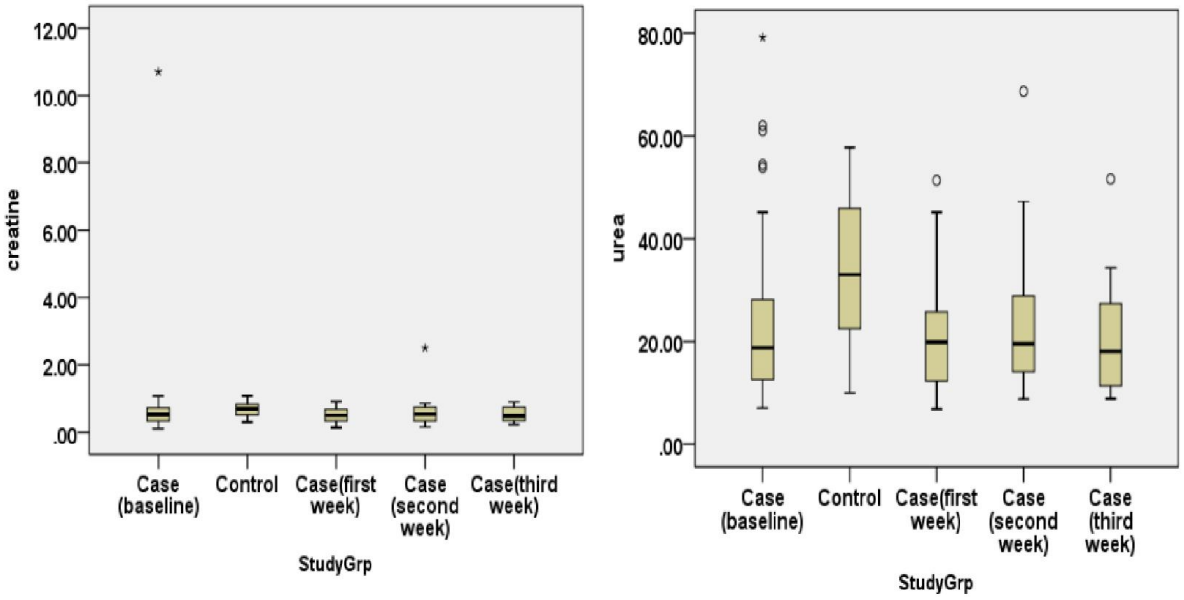

Figure 4. Serum creatinine (mg/dl) and urea (mg/dl) levels in burn patients in comparison to control and each successive weekly data.

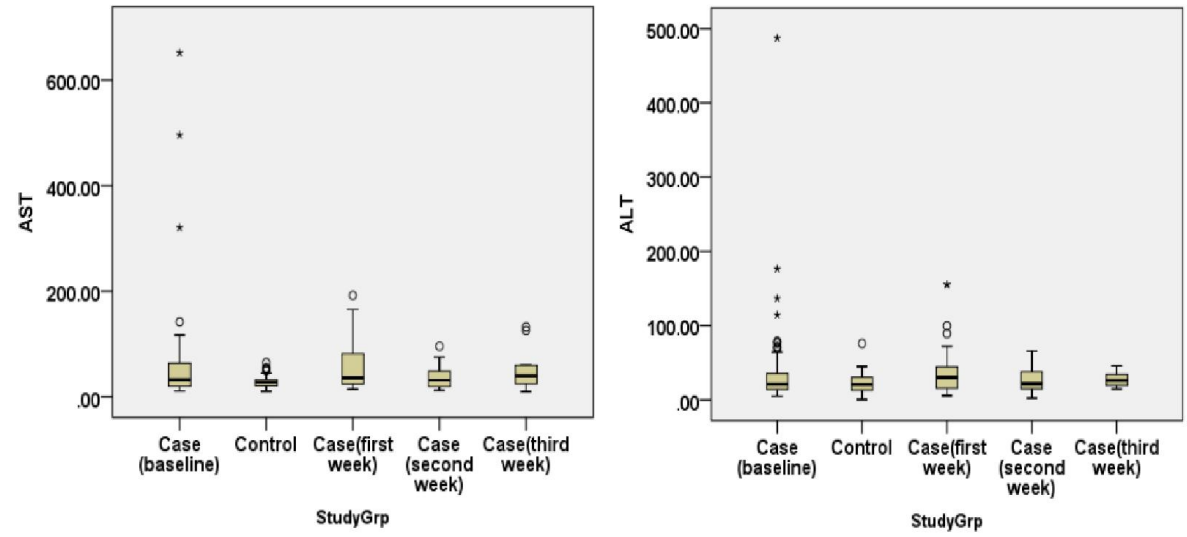

Figure 5. Serum AST (IU/L) and ALT (IU/L) levels in burn patients in comparison to control and each successive weekly data.

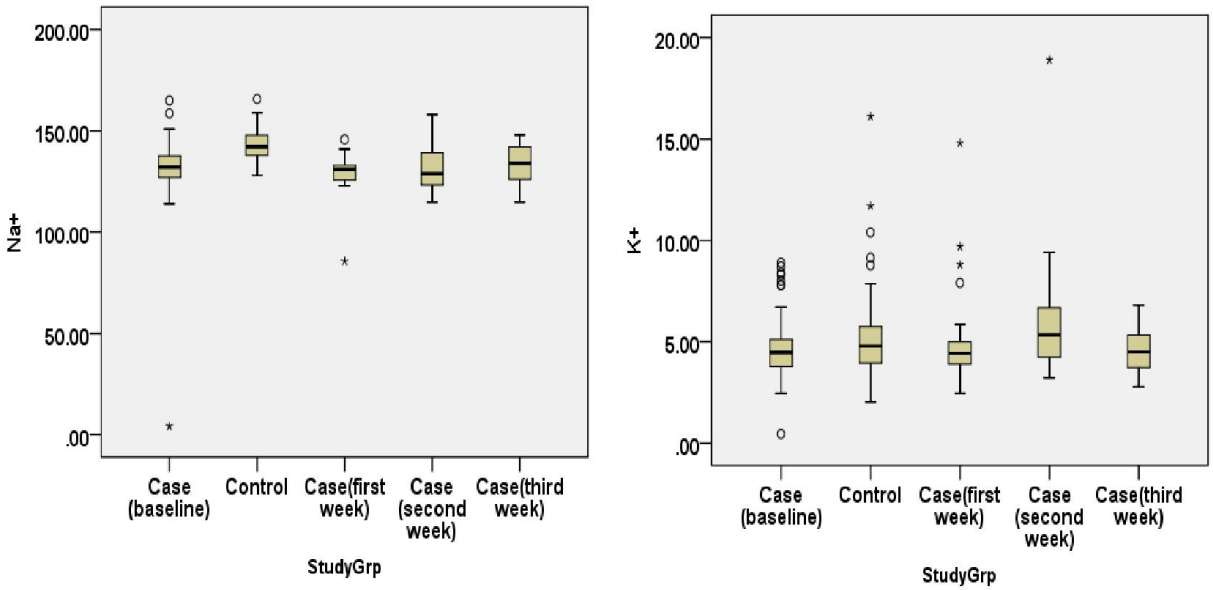

Figure 6. Na^{+} (Mmol/l) and K^{+}(Mmol/l) in burn patients in comparison to control and each successive weekly data.

5.4Association of Burn severity with Hematological change

High mean change in WBC count (10.7±4.40) was observed when Total Body Surface Area (TBSA %) burnt got more severe (<10% (TBSA), (and (>20 TBSA) (11.6± 4.78) (WBC). Whereas, the opposite was observed when total burned surface area increased there was a decrease in mean platelet count, in the lowest category (<10% TBSA) it was (415.91±151.62) and in >20% TBSA category it decreased to (348.12 ± 165.94). In the case of mean change in RBC and HGB concentration, there was a similar change seen from <10% to >20 % (TBSA); while under <10% (TBSA) in RBC it was (4.58±1.14) and in HGB it was (13.15±3.73); in 10-20% (TBSA) category RBC decreased to (4.37±1.11) in the same way as HGB also decreased to (12.31±3.13); but in the last highest value category (>20% TBSA) both increased (HGB (12.51±2.74), RBC (4.44±1.06) (Table.6).

Table 6. Association of burn severity with Hematological change of burn patients atYekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

burned surface area		WBC	RBC	HGB	PLT
<10	Mean	10.7041	4.5821	13.1579	415.9167
	N	37	38	38	36
	Std. Deviation	4.40694	1.14482	3.73925	151.62084
10-20	Mean	11.0411	4.3742	12.3138	392.4860
	N	54	55	58	57
	Std. Deviation	4.55893	1.11871	3.13813	120.16092
>20	Mean	11.6884	4.4411	12.5175	348.1277
	N	43	47	48	47
	Std. Deviation	4.78814	1.06270	2.74324	165.94743

5.5 Association of Burn severity with some of selected Biochemical Parameters

The results in this study showed that as TBSA burn got higher in burn patients there was also a higher mean change in serum urea and AST concentration. We can see that in this two markers, the highest mean change is seen in the highest TBSA category (>20%) where urea was (26.53±16.98) and AST (58.39±72.08). Serum creatinine and ALT concentrations also showed a similar change, both decreased from <10% to 10-20% (TBSA) while highly increased from 10-20% to >20% (TBSA) (Table.7)

Table 7. Association of burn severity with selected biochemical parameters of burn patients at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

TBSA		urea	creatine	AST	ALT
<10	Mean	19.5768	.5905	46.1763	33.4289
	N	38	38	38	38
	Std. Deviation	9.33004	.38426	38.94562	29.73927
10-20	Mean	21.0083	.5050	56.7860	30.7828
	N	58	58	58	58
	Std. Deviation	10.91716	.20875	92.39797	32.10401
>20	Mean	26.5327	.7308	58.3988	42.8427
	N	48	48	48	48
	Std. Deviation	16.98502	1.48784	72.08808	70.29206

5.6Association of Burn severity with Selected Electrolyte

A high mean change was seen in serum Na^+ concentration in the lowest burn surface area (<10%) (TBSA); whereas in serum K^+ concentration it was seen in the largest category (>20%) (TBSA). The lowest mean change of serum Na^+ concentration was seen in 10-20% (TBSA) and that of serum K^+ concentration was seen in <10% (TBSA) (Table 8).

Table 8. Association of burn severity with electrolytes of burn patients at Yekatit12 and AaBET hospital, Addis Ababa, Ethiopia from October, 2020 to July, 2021.

TBSA%		Na+	K+
<10	Mean	133.2132	4.6513
	N	38	38
	Std. Deviation	10.25502	1.29297
10-20	Mean	128.2453	4.9279
	N	58	58
	Std. Deviation	19.02525	1.74270
>20	Mean	131.8723	5.3352
	N	48	48
	Std. Deviation	9.06556	2.90646

6. Discussion

In our study from 72 burn patients majority of them were female. Most of the patients were from urban and majorities were children (1-15 years old), whereas in control group most of them were adult (>30 years) And scald burn were the most recorded causative agent. Second degree of burn injury were high than other degree. Leukocytosis was seen during admission and RBC after initial increment there was a decrement. Serum creatinine and urea were decreased only during second week while increased on admission, first week and third week. Serum transaminases (AST and ALT) were initially increased. On admission day analyses both K^+ and Na^+ were decreased.

During study period there were fluctuations of patient number from week to another week, due this we failed to discuss the changes might seen in all patients starting from admission day to third week. During admission time all 72 burn patients were described, on first week from 72 patients there were 32 patients, on second week from 72 burn patients there were 26 and on third week 14 of burn patients were found from 72 total burn patients.

The current study revealed that burn cases were comparable between females and males 37 (51.4%) and 35 (48.6%) respectively. This is in concordance with the study done in Egypt which reported from a total of 40 burn patients 23(57.5%) were women and 17 (42.5%) were men (20). This is explained by the fact that women do most of the kitchen work especially cooking in the Ethiopian culture. Therefore, this exposes them to a higher risk of burn injury.

In the current study, the majority of the burn injuries 32(44.4%) were occurring in children. This finding was similar to studies done in Ghana (41.1%) (17), Asmara, Eritrea (75%) (21), Yekatit 12 Hospital, Addis Ababa (76%) (22) And western Kenya (82%) (23). The reason might be due to children being unaware of the surrounding environment, lack of instinct to understand the danger of different objects, high physical activity and exposure to hazardous situations both at home, school, and play grounds.

The current study revealed that Scald 31(43.1%) to be the common cause of burn injuries followed by Flame 27(37.5%) and least cases were caused by Electrical 14 (19.4%). A similar finding was reported in Jimma, Ethiopia (24) which revealed that scald (47.6%) to be the common cause of burn injuries followed by flame (29.8%). This finding is also in line with many other studies done in different countries; USA (25) Iran (26), Italy (27) China (28). The reason could be that scald burn mostly occurs in the home, mostly caused by hot water, boiling water, hot tea in the kitchen during meal preparation (24).

In case of Burn degree, this study showed that Second degree burn outnumbered Third degree burn. This is in line with the study done in Yekatit 12 hospital, Addis Ababa where the majority of the burns (56.5%) were second degree burns and the rest (43.5%) were third-degree burns (22). A study done in Brazil also reported that Second degree burn to be predominant (62.6%) (29). this finding is also consistent with the other previous studies done as well (21, 23, 26, 28, 30, 31). This might be explained by the common cause of burn injuries in this study was scald burn, which affects the outer layers of skin (epidermis) and part of the second layers of skin (dermis).
In our study, the total body surface area (TBSA%) affected in most of the patients was in (10-20%) category. This finding is similar to other previous studies (4, 21, 24, 30). This might be due to similarity in sample size, admission criteria of burn injuries, causative agent and body loss surface epithelial that increase TBSA of burn patients (24).

With regard to hematological parameters, Leukocytes were significantly increased on admission day and then decreased in a non-significant manner during 1st, 2nd and 3rd weeks. This finding is in line with the study done in Egypt that reported Leukocytes to be high on first day (7). It also agrees with the study done in Korea which reported high increases of leukocyte count which was

seen on the first day reaching peak in 12 hours after injury and then began to decrease (14). This finding is also similar with regard to leukocyte with study done in India which reported that leukocytosis developed immediately after burn (32). In all cases WBC was elevated on average on admission day probably due to the initial initiation of systemic inflammation causing mobilization of leukocytes (33). WBC then starts to decrease over the next few days after admission.

Regarding RBC we found that it was non-significantly increased on admission day and first week and then non-significantly decreased in second week and third week. HGB were non-significantly lowered on admission day and increased in first week and second week, lastly on third week it got decreased. This finding agreed with study done in India which reported initially there was increment of HGB and HCT level and progressively decreased (1). And there is also similarity in finding with regard to RBC in Study done in Egypt which reported a rise in RBC level on the first day and got lower in successive days (7). However, there was contradiction with finding studied in Korea that reported a high level of HGB immediately after burn which reached its peak within 12 hours and got lower on the following day (14). The loss of red blood cells in the first few days following a severe burn is likely due to hemolysis of red blood cells (34). Following this early period, alterations in the bone marrow production of erythroid lineages probably results in the persistence of low numbers of circulating red blood cells reflected in low HGB and HCT (35).

In case of Platelet it was significantly increased on admission day then decreased in a non-significant manner during 1^{st}, 2^{nd} and 3^{rd} weeks. This study is in line with study done in Korea which stated that Platelet increased immediately after burn and decreased gradually (14). It is also similar with finding of study done in Egypt which stated platelet level showed a significant decline over the study period (7). A study done in USA also showed that PLT decreased early following a severe burn injury. By the 4th day after injury the mean PLT was a little over 50% of the admission level (12). The decrease in platelet count was caused first; burn injury may cause a change in platelet kinetics causing an increased migration and utilization of circulating platelets (36, 37). Second, following a severe burn, an increased level of fibrinogen may be present causing increased platelet aggregation which can result in a decrease in plasma levels of platelet

(38). In general, the decrease in platelet count was caused by multiple factors, increased Platelet destruction, hemodilution and reduced platelet production (39).

The results show that serum Creatinine level non-significantly decreased on admission day, 1st and 2nd week when compared to the control, admission day's record and first week record respectively. The same trend was seen in serum Urea level too, the only difference is on admission day in case of serum Urea level it was significantly decreased. This finding is in line with the study done in Ghana which stated that in all age categories there were low levels of serum creatinine and urea concentration (17). The lower serum urea and creatinine levels in the burnt patients is explained by Bonate, (1990) (40) who stated that during the hypermetabolic phase in thermal burnt patients, beginning 48 hours after the thermal injury, an increased cardiac output is observed as a compensatory mechanism, with a concomitant increase in blood flow to the kidneys and liver. Consequently, there was an increase in the glomerular filtration rate (GFR), increasing creatinine clearance which may explain the lower serum urea and creatinine levels in the burnt patients.

Regarding serum AST levels, there was increment on first day (significantly) and third week (non-significantly) and decreased in first and second week non-significantly. With regard to serum ALT levels, there was a significant increment on admission day, non-significant increment at 1st week and 3rd week and there was a decrease on 2nd week. As we can see there was an elevation of these enzymes on admission day which agreed with study done in Egypt that concluded that there was a high elevation of liver enzyme with high record on 5th day (7) and also have similarity with finding of study done in India which reported that there were a significant increment of AST and ALT because of an accumulation of edema (1). After burn injury there will probably be an accumulation of hepatic edema which can be a cause for liver damage, due to this there will be a releasement of liver enzymes which is a principal indicator for hepatocyte injury. In normal state these enzyme are present in low concentration, but if there is hepatocyte cell injury or an alteration to the permeability of cell membrane, then these enzyme will be released to circulation (41).

In case of serum Na^+ electrolyte levels, the present study showed that there was decrease on admission and 1st week and increased non-significantly on 2nd and 3rd weeks. This result is consistent with the study done in in Egypt by Saleh S.M (7) which reported that there was a significant Na+ decrease in serum level on the 3rd and 5th days post burn while its level showed non-significant decrease on admission day. The low Na+ occurred due to an increase in vascular permeability increased interstitial osmotic pressure in burn tissue and cellular edema with most significant shifts occurring in first hours. Hence, Hyponatremia is frequent and the restoration of sodium losses in burn tissue is therefore essential (15, 42).

With regard to serum K^+ concentration, it decreased (Hypokalemia) on admission and last week (in our case 3rd week) but there was an elevation (hyperkalemia) on 1st and 2nd weeks. While a study done in Egypt reported that serum K+ mean value showed non-significant increase during the 1st day post burn however, it showed a significant decline on the 3rd and 5th days post burn (7). The study done in Ghana also reported that serum potassium levels were significantly high on ≥ 2, 7th and 14th days post thermal burns in all patient categories as compared to controls. However, there was a decline in the potassium levels on the 14th day post burns period (17). Kaddoura *et al.* (2017) (43), Al-Muhammadi and Azeez (2011) (44) also reported that significant hyperkalemia occurred early in burn patients prior to resuscitation therapy. In general, hypokalaemia is experienced after stress states and has been attributed to a combination of the effect of adrenaline and insulin. Adrenaline stimulates receptors on skeletal muscle with consequent uptake of potassium from the circulation. It may be possible that total body potassium is not reduced. The hypokalaemia experienced by burnt patients after resuscitation could be due to increased potassium losses through urine, gastric, fecal and the intracellular shift of potassium because of the administration of carbohydrates (42).

Whereas, in major burns the initial resuscitation period (between 0 and 36hrs) is characterized by hyperkalaemia(44). The hyperkalaemia experienced at this stage has been attributed to massive tissue necrosis (42).

The severity of Burn injury is mainly dependent on the depth and body surface area it injured. In study done in Canada, Burn severity is calculated or can be known by total body surface area it got contacted by injury, in this finding burn injury that was found in above 20% was considered

as severe burn injury and was responsible for hypermetabolic reaction (45). So in our case, we categorized TBSA into three categories and tried to see its association with change of hematological parameters. In case of Leukocyte (TBSA=WBC <10%=10.7, 10-20%=11.04 and >20%=11.7) there was a direct relationship with TBSA. As burned surface area got high there was a high mean change in leukocyte. This showed that there was an association between the two.

With regard to blood RBC and HGB level, there was depletion when it went from <10% to 10-20% but in >20% it increased slightly. Serum Platelet level was in inverse relationship with TBSA, as TBSA increased serum Platelet level decreased, this can help us to conclude that as the burn injury becomes severe the level of thrombocytopenia increases. In the study done in Croatia (25) there was some similar finding with our findings, it reported that there was a significant thrombocytopenia only in severely injured burned patients (severe burns, > 10% TBSA). Of the many possible causes for the occurrence of thrombocytopenia, platelet destruction and bacterial sepsis may be the case. This brings us to the conclusion that, as the burn size increased there would be pronounced change in Hematological profile. This finding is in line with study done in Korea which stated that the bigger burn size, it resulted in bigger leukocyte, hemoglobin and platelet count change (14).

With regard to serum urea level, there was an elevation when we went from low burn size to high, which implied that high burn size resulted in high change of blood urea. In case of second markers of renal function, in our case which is serum creatinine level increased from TBSA 10-20% to >20% but it decreased from <10% to 10-20%. There was slightly high change in the highest TBSA category which is >20%. This means that in a severe burn injury, there will be a high level of serum creatinine and Urea as our study revealed. According to the study done in Egypt, burn was a major cause for reducing blood flow to kidney which brought an accumulation of nitrogenous waste product such as urea and creatinine, this led to kidney failure. In this study they concluded that an acute kidney failure depended on burn size and depth which is in agreement with our findings (20).

In case of transaminase liver enzyme, serum AST mean change was high as we went from lowest TBSA to the highest percentage, which showed that as burn size got wider, serum AST level also increased. In case of serum ALT, from the three categories there was a high mean change in the largest burn size which was >20% and the lowest was recorded in 10-20% category. Other studies show that the severity of thermal injury has no relation with serum enzyme levels at the onset whereas in the delayed phase, serum enzymes are better correlated with the severity of the injury (46). On the other hand, Mozingo *et al.* (2008) (47) reported that there was a tremendous increase in the hepatic aminotransferase enzyme level during the first week in patients with burn greater than 50% TBSA. This might be explained by severe tissue damage of the skin after burn injury and lipid peroxidation process (46).

In the case of serum K^+, there was a slight elevation as burn size got bigger; a bigger change was recorded in the highest burn size category. This pointed that, as burn size got larger there was also a high change in serum K^+ level. In contrast to our finding, a study done in Colombia found association between the serum potassium concentration and either %TBSA burned (48). In case of serum Na^+, the biggest change was seen under the lowest burn size (<10%) and secondly under the largest burn size (>20%).

7. Strength and limitation

7.1 Strength of the study

This study has strength with related to addressing all age categories. We have also measured the profiles on multiple point of time. Moreover, we have also recruited control groups to scrutinize and compare the relative values of the different hematological and biochemical profiles.

7.2 Limitations of the study

The main limitation of this study was the high cost of biochemical analyses which limited the number of biochemical parameters that were analyzed.

8. Conclusion and Recommendation

8.1 Conclusion

Burn injury occurred more in female than in male. Types of burn caused injury higher than other were scald burn and second degree burn were the highest case recorded. Burn injuries resulted in disturbance of hematological and biochemical profile which in turn complicate physiological parameter of patients. This fact asks health care group to estimate the hematological and biochemical profile so as to expect the prognosis in burn patients and body response to the different therapeutic lines.

8.2 Recommendations

Based on our findings we suggest more large scale studies that determine the levels of hematological and biochemical parameters as predictors of prognosis in burn injuries. We recommend strict patient follow up for longer time for those severely injured and showed altered hematological and biochemical parameters.

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10. Annexes

Annex I. Participant Information sheet (English Version)

Participant Information sheet

Addis Ababa University College of Health Sciences Department of Laboratory Sciences

Principal Investigator: Ashenafi legese

Purpose: To evaluate what changes of Hematological and Selected biochemical profiles of burn patients happen and to give clue for proper patient managements by based on a sample of patients visiting Yekatit 12 Hospital and Addis Ababa Burn, Emergency and Trauma Hospital (AaBET Hospital), Addis Ababa, Ethiopia.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving blood samples.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study.

Benefits of the study: There will be no financial benefit to you. But the result of the study will be used for your clinical care as well as plays a role in the Burn injuries managing and control program. There will be no compensation for using your blood sample.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind.

Your withdrawal of consent will not affect your right to receive medication

Annex II. Consent form for adults (≥18 years) (English version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that I would participate in this study t**o collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Name_____ Date _____ /____ /____ (dd/mm/yy)
Signature_____

If illiterate;

Name _____ Date ____/____/_____ (dd/mm/yy)
Signature_____
Phone number_____

Name of Researcher _____ Date _____/____/_____
(dd/mm/yy)
Signature_____

Annex III. Consent form for parents/guardians (English Version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that my child participates** in this study provided he/she gives assent to collect her/his blood and be a participant in this study and understand that I have the right to withdraw my child from the study at any time.

Name _____ Date ____ /____ /____ (dd/mm/yy)

Signature_____

If illiterate;

Name _____ Date ____ / ____ /_____ (dd/mm/yy)

Signature _____

Researcher Name _____ Date _____ /____ /_____ (dd/mm/yy)

Signature_____

Annex IV. Assent form for children aged 12-17 years (English Version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily assent that I would participate in this study provided my parents/guardians give their consent** to collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Name _____ _____Date ____ /____ /____ (dd/mm/yy)

Signature _____

If illiterate;

Name of literate witness _____ Date ____/____/_____ (dd/mm/yy)

Signature_____

Phone number (parents/guardians)_____

Name of researcher,_____ date _____/____/_____ (dd/mm/yy)

Signature _____

If you have any question you can ask the following individual

Ashenafi Legese

Department of Medical Laboratory Sciences

Cell phone: +251-9 20 67 40 77 E-mail:- 1ashenafilegese@gmail.com

Annex V. Participant information Sheet (Amharic version)

የተሳታፊዎች መረጃ ቅጽ

ጥናቱን የሚያጠናው፤ አሸናፊ ለገሠ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ ዓላማ

የጥናቱ ዓላማ በቃጠሎ አደጋ የተጎዱት ህመምተኞች በጉዳቱ ሳቢያ በ hematological parameters እና selected biochemical parameters ላይ ልመጣ የሚችለዉን የላብራቶሪ ምርመራ በማድረግ ማወቅ ሲሆን ይህም ለህመመተኞቹ ተገቢ ህክምና ለማግኘት እገዛ ስላለዉ ይህን ጥናት ማድረግ አሰፈላጊ ሆነዉ ተገኝተዋል፡፡ ይህም ጥናት የሚካሄደዉ በ የካቲት 12 ሆስፒታልና በአበት ሆስፒታልነዉ፡፡

በጥናቱ ወቅት ከእርስዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ የደም ናሙና መስጠት ነው፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘት ስለሚረዳ በቂ የሆነ ህክምና ለማግኘት ይረዳል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜም አይኖርም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ ላይ ያለ መሳተፍ መብት አለዎት፡፡

ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼ ያለሁ፡፡

ፊርማ _____

መረጃውን የሰበሰበው ግለሰብ ስም_____

ፊርማ_____

የዋና ተመራማሪው አድራሻ፤

- አሸናፊ ለገሠ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤናሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ
- **ኢ-ሜይል**፡፡1ashenafilegese@gmail.com **ስልክ** ፣ +251-920 674077

Annex VI. Consent form for adults (≥18 years) (Amharic version)

ከላይ ያለዉን መረጃ አንቢብያለዉ ወይም ተነቦልኛል፡፡ በዚህም ላይ ያለኝን ጥያቄዎች ለመጠየቅ ዕድል አግኝቻለዉ ላቀረብኩት ጥያቄዎችም አጥጋቢ መልስ አግኝቻለዉ፡፡ ስለዚህም የደም ናሙናን በመስጠት በጥናቱ ዉስጥ ለመሳተፍ ሙሉ ፍቃደኛ መሆኔን እገልጻለዉ ፡፡ እንዲሁም በየትኛዉም ጊዜ ከጥናቱ እራሴን ማግላል እንደምችልም ተረድቻለዉ፡፡

የተሳታፊ ስም፡ _____ ቀን_____/____/_____

ፊርማ፡-_____

ማንበብ/መስማት ለማይችሉ

ስም፡-_____ ቀን_____/_____/_____

ስልክ ቁጥር፡-_____

ፊርማ፡-_____

የጥናቱ ባለቤት ስም _____ ቀን _____/_____/_____

ፊርማ፡- _____

Annex VII. Consent form for parents/guardians (Amharic Version)

ከላይ ያለዉን መረጃ አንብብያለዉ ወይም ተነቦልኛል፡፡ በዚህም ላይ ያለኝን ጥያቄዎች ለመጠየቅ ዕድል አግኝቻለዉ ላቀረብኩት ጥያቄዎችም አጥጋቢ መልስ አግኝቻለዉ፡፡ ስለዚህም ልጄ በዚህ ጥናት ላይ የደም ናሙናን በመስጠት ለመሳተፍ በዉክልናዬ መሠረት ፈቃደኛ መሆኔን አገልጻለዉ፡፡ አንዲሁም አሰፈላጊ ሆነዉ ሲገኝ በየትኛዉም ጊዜ ከጥናቱ ማቋረጥ እንደምችል ተረድቻለዉ፡፡

የተሳታፊ ስም፡ _____ ቀን_____/____/_____

ፊርማ፡-_____

ማንበብ/መስማት ለማይችሉ

ስም፡-_____ ቀን_____/_____/_____

ስልክ ቁጥር፡-_____

ፊርማ፡-_____

የጥናቱ ባለቤት ስም _____ ቀን _____/_____/_____

ፊርማ፡- _____

Annex VIII. Assent form for children aged 12-17 years (Amharic Version)

ከላይ ያለዉን መረጃ አንቢብያለዉ ወይም ተነቦልኛል። በዚህም ላይ ያለኝን ጥያቄዎች ለመጠየቅ ዕድል አግኝቻለዉ ላቀረብኩት ጥያቄዎችም አጥጋቢ መልስ አግኝቻለዉ። እኔን መወከላቸዉን በሰጠዉ ማረጋገጫ መሰረት በዚህ ጥናት ላይ የደም ናሙናን በመሥጠት መሳተፍ እንደምችል ቤተ ሰቦቼ ሙሉ ፍቃዳኛ ናቸዉ። አንዲሁም አሰፈላጊ ሆነዉ ሲገኝ በየትኛዉም ጊዜ ከጥናቱ ማቋረጥ እንደምችል ተረድቻለዉ።

የተሳታፊ ስም፡ _____ ቀን_____/____/_____

ፊርማ፡-_____

ማንበብ/መስማት ለማይችሉ

ስም፡-_____ ቀን_____/_____/_____

ስልክ ቁጥር (የቤተ ሰብ)፡-_____

ፊርማ፡-_____

የጥናቱ ባለቤት ስም _____ ቀን _____/_____/_____

ፊርማ፡- _____

ማንኛዉንም ጥያቄ ካሎት ከታች የተጠቀሰዉን ግለሰብ መጠየቅ ይችላሉ።

አሸናፊ ለገሠ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ

ስልክ ቁጥር፡- 0920674077

Annex IX. A. Data collection format for Socio-demographic and Clinical Information Data of Burn Patients

Date /____/_____/

Code number----------

Hospital no---------- Address ---------- Tele {phone number}----------

Age _____Sex_____

Living Area: Urban_____ Rural_____

The time of the burn----------

Burn types:-

Heat----------

Electricity----------

Hot liquid (scalds) ----------

Chemicals----------

Burn degree:-

First----------

Second ----------

Third ----------

Surface area affected:-

_____

Annex IX.

B. Data collection format for Socio-demographic Data of Control groups

Date /____/_____/

Code number----------

Hospital no---------- Address ---------- Tele {phone number}----------

Age _____Sex_____

Living Area: Urban_____ Rural_____

Annex IX.

C. Data collection format for Laboratory Data

Date of sample collection _____day_____ Month _____year

Time of sample collection _____ before treatment after treatment

Total no of sample received _____

Results:

a) Completed ☐ b) Incomplete c) Excluded

Action taken for the incomplete data_____

Rejected sample

Clotted ☐ Hemolyzed Others

_____

Unlabeled

Insufficient

Test Results:- CBC

Id	WBC	RBC	HGB	HCT	PLT

- Test Results:- RFT

ID	Urea	Creatinine

- Test Results:- LFT

ID	AST	ALT

- Test Results:- Electrolyte

ID	Na^+	K^+

Date and signature of laboratory technician _____

Comment:_____

If you have any question you can ask the following individuals

Ashenafi legese

Department of Medical Laboratory Sciences

Cell phone: +251-9 20 67 40 77

E-mail:- 1ashenafilegese@gmail.com

Annex X. Dummy tables

Table 1. Burn characteristics of patients at Yekatit 12 hospital and AaBET hospital

Burn characteristics	No	%
Cause of burn		
Flame		
Hot liquid		
Electric current		
Degree of burn		
First		
Second		
Third		
TBSA% (Total body surface area)		
<10%		
10-20%		
>20%		

Table 2. Age and Sex distribution of Burn patients and control groups at Yekatit 12 hospital and AaBET hospital

Variables	Frequency	Percent
Sex		
Male		
Female		
Total		
Age		
Children		
Adult		
Older		

Table 3. Hematological parameters of burn patients and Control groups.

Variables	Male	Female	Total	P
RBC				
Hemoglobin (g/dL)				
Hematocrit (%)				
WBC ($x10^6$/L)				
PLT ($x10^3$/L)				

Table 4. Selected Biochemical profile of burn patients and control groups.

Variable	Male	Female	Total	P
RFT				
Urea				
Creatinine				
LFT				
AST				
ALT				
Electrolyte				
Na+				
K+				

Annex XI. SOPs for Different Laboratory Procedures

A. SOPs for Blood Sample Collection

Identify and prepare the patient where it is adult and conscious follow the steps outlined below. Compare the data with the information on the request form and report any major discrepancy to the responsible person in the area.

- ✓ Introduce yourself to the patient, and ask the patient to state their full name and demographic information
- ✓ The patients should have an order or requisition slip for the test to be performed
- ✓ Check the test order form, all the records must be completed.
- ✓ If the patient has any drug therapies it may affect the test, and the test ordered physician be responsible for advising his/her patient if the requirement to discontinue certain drugs.
- ✓ Ask whether the patient is latex sensitivity if so use non-latex supplies where appropriate
- ✓ Ask whether phobias or has ever fainted during previous injections or blood draws.
- ✓ If the patient is anxious or afraid, reassure the person and ask what would make them more comfortable.
- ✓ Make the patient comfortable in a supine position (if possible).
- ✓ Discuss the test to be performed and obtain verbal consent. The patient has a right to refuse a test at any time before the sampling, so it is important to ensure that the patient has understood the procedure.

Patient Reassurance

- Describe procedure to the patient
- Get oral consent from the patient
- Tell the patient it is going to be slightly painful
- Gain patient's confidence
- Refuses - Never force a patient.
- Describe the use of the study to the patients.

Assemble supplies and position patient

- Inspect all supplies for possible defect and applicable expiration dates.
- For patient safety, draw all specimens with the patient seated comfortably in an appropriate chair or by lying down.

Each specimen must be clearly labeled with the following:

- 2 patient identifiers, usually patient name and hospital or clinic number
- Type of specimen

Note: labels should always be placed on the specimen bottle, tubes, etc., not on the lid

Each specimen must be accompanied by a requisition form. The requisition form must include the following:

- 2 patient identifiers, usually patient name and hospital or clinic number
- Patient age, sex and address (hospital ward, clinic, etc.)
- Date and time collected
- Name or initials of collector
- Test requested
- Name of requestor
- Any antibiotics patient may be on
- Any other pertinent medical information

Apply Tourniquet

- Tourniquet is used to increase intravascular pressure, which facilitates vein palpation and filling of the tube (s) or syringe.
- Tourniquet application should not exceed one minute as localized stasis with hemo-concentration and infiltration of blood into tissue can occur.
- If the patient has a skin lesion at the intended tourniquet location, consider an alternative draw site, or apply the tourniquet over the patient's gown (cloth)
- Wrap the tourniquet around the arm 3-4 inches (7.5-10 cm) above the puncture site.
- Ask the patient to form a fist, but avoid vigorous hand exercise.
- Collect blood form median capital (H pattern) and median (M pattern) veins because these veins are typically closer to the surface of the skin, more stationary, less painful upon needle insertion, and less likely to injure nerves if needle placement is not accurate.

Put On Gloves

- The phlebotomist must put gloves on before the veni-puncture is performed with consideration for latex sensitivity as discussed.

Cleanse vein-puncture Site

- Use a gauze pad with 70% isopropyl alcohol solution, or a commercially prepared alcohol pad.
- Cleanse the site with a circular motion from the center to the periphery.
- Allow the area to air dry.
- If the vein-puncture proves difficult and the vein must be touched again to draw blood, the site must be cleansed again.

Vein-puncture procedure using needle and syringe

- Break the seal and look for any defects, check the plunger.
- Prepare the patient by informing him/her that the vein puncture is about to occur.
- With the bevel up, puncture the vein with the needle at angle of insertion of 30 degrees or less.
- Keeping the needle as stable as possible in the vein, slowly withdraw the desired amount of blood and ask the patient to open his arm.
- Release the tourniquet as soon as possible, after the blood begins to flow.
- Transfer the blood from the syringe to a venous blood collection tube by piercing the stopper with the needle. Allow the tube to fill without applying pressure to the plunger until flow ceases. This will helps to maintain the correct ratio of blood to additive if an additive tube is being used.
- Mix the additive tubes by inversion. Do not shake tubes. Rubber stoppers should not be removed from venous blood collection tubes to transfer blood to multiple tubes.

Additional Considerations

Haemolysis

To prevent haemolysis; allow the vein-puncture site to air dry after cleansing, never draw blood through a hematoma & make sure the needle is fitted securely on a syringe to avoid frothing.

Hematoma

To prevent hematoma, the phlebotomist should make sure the needle fully penetrates the uppermost wall of the vein, remove the tourniquet before removing the needle, use the major superficial veins, apply small amount of pressure to puncture site.

Materials and Supplies required for the collection, storage and shipment of whole blood/Plasma/Serum

1. 10% bleach (0.5% sodium hypochlorite) in spray bottle disinfectant

Annex XI.

B. Standard Operating Procedure (SOP) for Hematological parameters that will be analyzed by Beckman coulter

CBC Analysis

The complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells, and platelets.

Method

The Coulter Principle accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

Sample Preparation

The aspiration pump activates and aspirates 165 µL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors.

With each cycle, the BSV directs the delivery of sample and DxH Diluent to the WBC and RBC triple aperture baths. The RBC diluent and WBC diluent/Lyse dilutions enter through a port in the bath that is located at the bottom and tangential to a sloping surface for bubble free delivery and mixing. In the WBC bath, ~6.0 mL of DxH diluent and ~28 µL of sample are combined with ~1.08 mL of DxH Cell Lyse for a final dilution of 1:251. In the RBC bath, ~10 mL of DxH diluent and ~1.6 µL of sample are combined for a final dilution of 1:6250.

Detection/Sensing

After the mixing and incubation of sample and reagents, 6 inches of vacuum and aperture current are applied to the apertures simultaneously for the measurements of cell count and cell volume. The RBC and PLT count includes the application of sweep flow to prevent the recirculation of cells behind the aperture. All pulses generated by the apertures are collected and sent to the

Signal Conditioner Analyzer Card for analog to digital conversion. The process provides the following raw counts and digital measurements to the System Manager:

• Time

• Volume (pulse peak amplitude)

• Count rate

• Wait time

• Pulse width

The System Manager processes the measurements. The process includes:

• Coincidence correction

• Voting

• The generation of 256 channel histograms for WBC, RBC, and PLT and their voting pattern analysis

• Interference correction

Pulse Editing

When cells pass through the aperture near the edge or at an angle rather than at the center, they create atypical pulses. These atypical pulses are excluded from analysis because they distort the true size of the cell. This prevents the atypical pulses from influencing size measurement.

Sweep Flow

The sweep flow is a steady stream of diluent that flows behind the RBC aperture during the sensing period. This prevents cells from re-entering the sensing zone and being counted as platelets.

Counting/Sizing

The RBC and WBC baths each have three discrete apertures that function as independent systems, utilizing the Coulter Principle to accurately count and size cells.

Coincidence Correction

Occasionally, more than one cell passes through the aperture at one time. When cells coincide, only one combined pulse is counted. As the frequency of coincidence is proportional to the actual count, the system automatically corrects results for coincidence.

Scaling

Scaling adjusts for calibration and reportable format.

Voting and Averaging

To prevent data errors due to statistical outliers or obstructions that may block an aperture, the System Manager votes on data for WBC, RBC, MCV, RDW, PLT and MPV, comparing data for all three apertures. The System Manager verifies that at least two apertures have produced data within an established statistical range of each other. A partial vote out of an individual aperture is not used in the averaged parameter value. Single aperture vote outs are tracked internally by the system and an error message is generated if an excessive level is reached. Partial vote out information is provided on the service log. When a parameter totally votes out, the system does not give any results for the affected parameter or for any parameters that are derived from it.

Hemoglobinometry

The lytic reagent used for the WBC prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobin to a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample. The accuracy of this method equals that of the hemiglobincyanide method, the reference method of choice for hemoglobinometry recommended by the International Committee for Standardization in Hematology.

After the WBC are counted, the lysed WBC dilution drains into the hemoglobin cuvette for Hgb measurement. Hgb is measured photometrically at 525 nm using the sample from the WBC analysis. A blank is introduced into the cuvette during each operating cycle. The Hgb blank provides a reference to which the sample signal is compared.

Generation of Histograms

The digital information from each WBC and RBC aperture is stored according to volume in 256-channel, size distribution histograms. Histograms show only the relative, not actual, number of cells in each size range.

IMPORTANT Do not estimate the number of cells from the distribution curves.

To ensure that the size distribution curves accurately reflect the true cell population, the sensing may be extended whenever the data accumulations are below a predetermined value. Double-clicking a histogram displays a larger view of the histogram. Each histogram is drawn in a black line and the area under the line is shaded as follows:

• WBC - light purple/lavender

• RBC - reddish orange/pink

• PLT - light green

IMPORTANT Histograms show only the relative, not actual, number of cells in each size range. Do not estimate the number of cells from the distribution curves.

VCSn Analysis

VCS Technology

The COULTER VCS established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity and laser-light scatter. The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the SPM into data plots.

Volume Analysis

Electronic Leukocyte Volume Analysis using low-frequency current has been used since 1967. It has been evaluated as a possible adjunct to the differential white cell count.

Conductivity Analysis

Cell walls act as conductors to high frequency current. The current, while passing through the cell walls and through each cell interior, detects differences in the insulating properties of the cell components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.

Light Scatter Analysis

Coulter's experience in flow cytometry dates back decades to Fulwyler's pioneering use of light scatter for cell analysis. Loken et al. and Jovin et al. discuss the relationship of particle size and refractivity to the angle of light scattered from a laser beam (19).

TTM and MTM

Historically, Beckman Coulter analyzers housed a flow cell in a Triple Transducer Module (TTM), first introduced commercially in the 1980s. The TTM flow cell was the location for detection of the processed samples. The TTM produced three measurement signals – volume, conductivity and light scatter. The DxH 800/DxH 600 replaces the TTM with the Multi-transducer Module (MTM), which measures additional multiple angles of light scatter, a major improvement over the single light scatter measured by the TTM. All Diff, NRBC, and Retic analysis occurs in the VCSn module. The VCSn module is responsible for controlled sample preparation and delivery of the prepared sample to the flow cell for analysis of the WBC

differential, reticulocytes and NRBC. The VCSn module includes the Air Mix and Temperature Control (AMTC) Module and the Multi-transducer Module (MTM).

Differential

Sample preparation occurs at the Diff mix chamber where sample and reagents are added in the following order: Diff Lyse, blood, additional Diff Lyse followed by an air mix. Next, Diff preservative is added, followed by a second air mix, and an incubation period The prepared sample is transferred to the MTM where cells are counted in an isometric sample stream. The algorithm analysis separates the WBC into five major populations.

NRBC

Sample preparation occurs at the NRBC Diff mix chamber where sample and reagents are added in the following order: Diluent, blood, additional Diluent followed by an air mix. Next, DxH Cell Lyse is added, followed by a second air mix, and an incubation period. The prepared sample is transferred to the MTM where cells are counted in an isometric sample stream. The algorithm analysis separates NRBC from WBC.

Reticulocytes

Reticulocytes are immature, non-nucleated erythrocytes retaining a small network of basophilic organelles, consisting of RNA and proto porphyrin. The enumeration of reticulocytes provides a simple, effective means to determine red cell production and regeneration. The most common means of measuring reticulocytes is to use supravital dyes, such as New Methylene Blue or Brilliant Cresyl Blue. These dyes precipitate and aggregate the basophilic substances within the reticulocyte, resulting in a granular, staining pattern easily seen with light microscopy.

Reticulocyte immaturity is related to cell volume and light scatter. Since more immature reticulocytes are larger, contain more RNA and cause increased light scatter, the cell volume and light scatter will increase with immaturity of the cell.

Sample Preparation

The sample preparation for Diff, NRBC, and Retic analysis occurs in the mix chambers in the AMTC module of the VCSn module. The blood samples used for analysis are delivered by the SAM and dispensed directly to the appropriate mix chamber. Next, the temperature-controlled reagents are delivered and the sample and reagents are mixed using a focused jet of air regulated to 4 psi. The mix chambers, reagents, and air are all temperature-controlled.

Detection/Sensing

Once the sample is prepared, the sample is delivered via the Distribution Valve (DV) to the MTM for sample detection. The MTM measures particle light scatter by utilizing a flow cell to pass particles through a sensing zone one cell at a time. The MTM flow cell measures volume, conductivity, multiple angles of light scatter, and axial light loss.

Volume and Conductivity

In the flow cell, low-frequency, direct current measures volume, while high-frequency (RF) current senses cellular internal content through measuring changes in conductivity.

Light Scatter Measurements

The MTM utilizes a flow cell to pass particles through a sensing zone one particle at a time and a diode laser to illuminate the particles. The illuminated particles both scatter and absorb a portion of the incident light. Sensors strategically placed around the flow cell collect the scattered light of interest. An additional sensor placed in the laser path measures the amount of light removed due to light scatter and absorption. This measurement is called Axial Light Loss.(18)**.**

Annex XI.

C. Standard Operating Procedure (SOP) for Biochemical Tests that will be done by Cobas C311 chemistry analyzer

PRINCIPLE:

The Roche Diagnostic Cobas C311 analyzer is automated, software-controlled analyzer for clinical chemistry analysis. It is designed for both quantitative and qualitative in vitro determinations using a large variety of tests for analysis. The Cobas C311 analyzer performs photometric assays and ion selective electrode measurements and uses serum/plasma.

PROCEDURE NOTES:

1. Reconstitute Activator (W2) if it has reached its 7-day expiration by warming to room temperature and reconstituting with 12 ml D.I. water and let set for 30 minutes.
2. Following the maintenance log perform the daily check

a. No disk (We will not have)

b. Check paper in printer

c. Check to make sure the water supply is on

d. Check for dripping of syringes

e. Check Cell covers and clean if necessary (in depth cleaning part of weekly)

f. Record water bath temperature (found on maintenance screen)

g. Visually check reagent levels on screen and expirations (for consumables and tests). If consumables are changed remember to reset volumes and prime reagent if necessary.

Always review Reagent screen to see when test cassettes are expiring. Ammonia needs to be conditioned for 24 hours before a new cassette is put on the analyzer, so when on board expiration is at 2 days we need to condition the Ammonia. To condition, open the gray cap only and set aside along with the c-pack in a light protected container that will allow airflow but restrict light and will not tip over. Document when the conditioning began to guarantee a full 24-hour conditioning. When the 24 hours is complete, return the gray cap to its original position and gently mix by circular motion. Put the c-back on the analyzer and calibrate immediately.

3. Perform hands on Daily Maintenance (Place analyzer in Maintenance Mode using Maintenance Mode button on right side of analyzer.)

a. Clean probes and shield pipe

b. Clean ISE sipper nozzle (when replacing sipper cover make sure shield doesn't touch either

cell cover)

c. Clean cell rinse nozzles

d. Clean ISE drain port

e. Take analyze out of Maintenance Mode

4. Place Sysclean (W1) and Activator (W2) in W1 and W2 slots on sample disk

5. Perform Daily Pipe (Push button on Maintenance log)

a. Daily maintenance

b. Pipe function

c. Daily pipes

6. Record photometer Check Values after Daily pipe (2 ways)

a. On the system Overview Screen press Print View button OR

b. Press Global Print, click on utility tab, under maintenance, press view.

7. Backup and clear information every morning.

a. Sample Data Clear

b. Backup and Clear

c. Check Mass Storage and Binary

d. File name is date (i.e. 10072015)

e. OK

8. Check the Preventative Action box on the Maintenance Screen

9. Reagent preparing

a. Reagent load/unload list

b. Yes

c. Print

d. Unload and load necessary c-packs

e. On Sunday morning unload all drug reagents, mix and reload

10. Calibration and QC select

a. Calibration

b. Recommended

c. Yes

d. Print

e. QC

f. Recommended

g. Yes

h. Print

i. Routine

j. Yes

k. Print

11. Remove check from Preventative Action box on the Maintenance Screen

12. Load samples in the appropriate slots on the sample disk

13. Hit Global Start and Start

14. Review Calibrations and QC

15. Place primary tubes or pour off tubes in patient positions on the outer row with the barcodes facing out.

16. To run a STAT sample while the instrument is running, press the Pause/Scan button, then pause and wait for the green light or simply wait for the green light and load the sample in the STAT positions on the inner row with the barcode facing inward.

17. Directions to print out selected reports when interface is down.

a. Highlight patient

b. Press the global print button

c. Highlight report under print format

d. Press Print

18. To retransmit the tests to the host

a. Go to Workplace

b. Data Review

c. Send to host

d. Send **(19)**

Annex XII. Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Ashenafi Legese (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor: Mikias Negash (MSc., PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mintewab Hussein (BSc., MSc.)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.