

Inflammatory Cytokines, Proteins, and White Blood Cells in Burned Patients Affected with Second and Third Degree of Burn

Dakhel Ghani Omran AL-Watify

¹*Collage of Science for Women/Department of Biology, University of Babylon, Hilla, Iraq, E-mail: dakhelghani@yahoo.com*

Abstract

Burns are associated with increasing metabolic activities of the body organs. High metabolism rates are initiated by activation of different inflammatory reactions and cellular mediators(cytokines). The present research was conducted to evaluate the inflammatory markers , proteins , and white blood cells in thermal burned patients . A total number of burned patients was 60 from both sexes (30 males and 30 females), then , the patients were classified according to severity of burn into four subgroups , the first burned male group involved 15 burned males affected with second degree of burn, and the second burned male group(15) was affected with third degree of burn . Similarly, the same classification was applied on burned females in this study . Thirty healthy subjects (15 males, 15 females) were selected as control groups. All ages of patients and controls were ranged between 2 5-35years old.

It was well found that the levels of C- reactive protein (CRP) were markedly increased ($p < 0.05$) in all patient groups in a comparison with their normal counterparts . Results of interleukin – 6 (IL-6) were significantly heightened ($p < 0.05$) in most burned patient groups except male group affected with second degree of burn ,since, they did not show a significant increase ($p > 0.05$). Concentrations of total serum protein , albumin , and globulin were tend to decrease significantly ($p < 0.05$) in most burned groups when compared with those healthy control groups.

Regarding total white blood cells (WBCs) count, data obtained from the present study indicated a significant increase ($p < 0.05$) of total WBCs in all burned patient groups as matching with control groups. Furthermore, differential WBCs explained the following abnormalities: lymphocyte levels were significantly decreased ($p < 0.05$) in all patient groups. Inversely, values of monocytes indicated an insignificant elevation ($p > 0.05$) in most burned groups except burned male group affected with third degree of burn which showed a significant increase ($p < 0.05$) in comparison with healthy groups. Statistical analysis of t-test indicated that granulocytes pointed out a significant elevation ($p < 0.05$)

of neutrophils level in all studied groups. Inversely, values of eosinophils were significantly reduced ($p < 0.05$) in most burned groups compared to healthy control groups. Significant and insignificant decreases were found in the levels of basophils of all burned groups when compared to those of control group.

Key words: Cytokines, Proteins, Burn, White blood cells, Inflammation.

الحركات الخلوية الالتهابية والبروتينات وخلايا الدم البيض لدى مرضى الحروق من الدرجة الثانية والثالثة

الخلاصة

ترتبط الحروق مع ارتفاع العديد من الفعاليات الايضية لمختلف اعضاء الجسم. ان ارتفاع العمليات الايضية تبدا من خلال تنشيط التفاعلات الالتهابية المختلفة والوسائط الخلوية . وظفت الدراسة الحالية لتقييم بعض العوامل الالتهابية والبروتينات وخلايا الدم البيض لدى الاشخاص المصابين بالحروق الحرارية . كان العدد الكلي للمرضى 60 شخصا مصابا بالحروق الحرارية ومن كلا الجنسين (30 رجلا و30 امرأة) , ثم صنف المرضى طبقا لشدة الحروق والجنس الى اربع مجاميع ضمت كل مجموعة 15 شخصا. كما شملت الدراسة اختبار اشخاص اصحاء (15 رجلا و15 امرأة) استخدموا كمجموعة سيطرة .

لوحظ حصول ارتفاع معنوي ($p < 0.05$) في قيم بروتين الطور الحاد لدى جميع مرضى الحروق عند مقارنتهم مع مجاميع السيطرة. كما بينت قيم تركيز الانترلوكين (IL-6) ارتفاع معنوي لدى جميع فئات المرضى ماعدا الدرجة الثانية, اذ انها لم تؤثر ارتفاع معنوي عند المقارنة مع الاشخاص الاصحاء. اما بخصوص قيم البروتين المصلي الكلي والابومين والكلوبيولين فقد اظهرت انخفاضا معنويا ($p < 0.05$) في جميع المجاميع المدروسة بالمقارنة مع الاشخاص الاصحاء .

اما بشأن العدد الكلي لكريات الدم البيض , فقد حققت ارتفاعا معنويا ($p < 0.05$) لدى جميع مرضى الحروق من الدرجة الثانية والثالثة عند مقارنتها مع الاشخاص الاصحاء. وفيما يخص العدد التفريقي لكريات الدم البيض, فقد تبين ان الجلايا للمفاوية قد انخفضت معنويا ($p < 0.05$) في جميع المجاميع واما الخلايا الاحادية فقد حققت ارتفاع غير معنوي ($p > 0.05$) في اغلب المرضى ماعدا الرجال المصابين بالحروق من الدرجة الثانية, فقد ارتفعت الخلايا الاحادية لديهم معنويا ($p < 0.05$). بينت التحليلات الاحصائية للخلايا الحبيبية وجود ارتفاع معنوي ($p < 0.05$) في جميع الاشخاص المصابين بالحروق. في حين لوحظ حصول انخفاض معنوي ($p < 0.05$) في قيم الخلايا الحامضية عند المقارنة مع المجاميع المدروسة. كما لوحظ ارتفاع معنوي ($p < 0.05$) وغير معنوي ($p > 0.05$) في قيم الخلايا القاعدية لدى مرضى الحروق عند مقارنتهم مع مجاميع الاشخاص الاصحاء .

الكلمات المفتاحية: الحركات الخلوية, البروتينات, خلايا الدم البيض, الالتهاب.

1 -Introduction

Burn resulted from thermal damage lead to defect so called coagulative necrosis to the tissues of skin and then subcutaneous tissues. the central zone of coagulation is surrounded by tissues which have a moderate levels of vascular damage lead to a drop in blood flow (tissue perfusion), stasis of blood to surrounding tissues lead to development specific areas called partial – thickness or full thickness wounds.

These abnormalities resulted because of production of chemical mediators, such as oxidants, C-reactive proteins, interleukins, and prostaglandins that are produced by the burned tissues, interaction among these mediators cause vasodilation for arteries and veins in affected area and subsequent aggregation of platelet causing stasis of blood flow [1]. Chemical mediators are also implicated in many disorders associated with burn which include inflammation, circulating disturbances, higher rate of metabolism, delay of healing. Tumor necrosis factor, interleukins, and gamma interferon are found to be associated with burn responses, it is well found that tumor necrosis factor (TNF) is responsible for neutrophil attraction [2] [3] [4] [5] [6]. Neutrophils play a key role in initiation of the microvascular damage and propagation the effects of burn in the stasis area, also, it is well documented that antibodies can be depressed neutrophil adherence and increase the blood flow in the zone of stasis [2]

2-Materials and Methods

3-Patients of the study:-

This research was completed at burn unit of Hilla teaching hospital in Babylon province. The total number of burned patients was 60 of both sexes (30 males and 30 females) and then sub-classified according to severity of thermal burn into four groups. First males group included 15 males affected with second degree of thermal burn and second males group (15) was affected with third degree of thermal burn. Also, burned females were classified into two groups, first female group (15) affected with second degree of thermal burn and second group of burned females (15) were affected with third degree of thermal burn. Thirty healthy subjects (15 males and 15 females) correspond with burned patients in their ages and gender criteria were selected to serve as a control group. All burned patients were selected according to diagnosis of consultant physicians specialized in unit of burn in that hospital. Ages of both burned patients and control subjects were ranged between 25 years to 35 years old. The burned subjects of this study (women and men) were non affected with chronic diseases and the information about normal health criteria were obtained by questionnaire of physicians and occasionally from patients themselves. Normal volunteers control groups (women and men) were no smoking, nonpregnant, no-contraceptive drugs, no cardiovascular diseases, free from diabetes, without thyrotoxicosis, and free from chronic diseases.

4-Collection of blood samples:-

All blood samples were collected at morning between 8-10 O clock in unit of burn from November 2015 to March 2016. Anticubital vein of left or right arm was selected to draw of blood samples after sterilized with alcohol solution (70%). The tourniquet was tightened around 7 Cm above targeted vein to ensure swollen of that vein. Asyringes of 23 Gauges were used to draw of blood. Two groups of tubes were prepared, the first of them with EDTA as anticoagulant to stop of blood coagulation and to complete leukocytes analysis directly. The second group of tubes were without anticoagulant (gel plain tubes) and they were used to prepare sera to be used for biochemical analysis. The blood samples in gel tubes were left at a least 10 minutes to coagulate of blood and then transferred to centrifugation at 3000 rpm.

The serum specimens were aliquoted to eppendorf tubes and kept at -20 c for future biochemical analysis.

5-Hematological tests

Total and differential white blood cells were obtained directly from Cell Dyn Ropy appa- ratus (Abbott Diagnostic, USA).

6-Determination of C- reactive protein:-

Microtiter strip coated with anti-c-reactive protein antibodies were already incubated with stander serum and tested serum . CRP is interacted to the surfaces of wells having anti- bodies against CRP during incubation period. A Washing procedure performed to ensure excluded unbound serum and then incubated with specific peroxidase conjugated anti- bodies. The absorbance length was at 450 nm (Demeditec Diagnostic GmbH company).

7-Determination of interlukin-6:-

IL-6 was determined by ELISA kit that based on standard sandwich enzyme- linked immune –sorbernt assay. The optical density of the final color was read at 450 nm (According to Boster Biological Technological Co.Ltd)

8-Determination of total serum protein:-

The peptide bonds of protein react with Cu^{+2} in alkaline solution to form complex color and the intensity of color is proportionate directly with total protein concentration in the sample and can be measured at 550 nm (Biolabo SA, company)

9-Measurement of serum albumin:-

Albumin in the sample binds with bromocresol green at acidic medium(pH 4.2)and it forms a complex color that proportionate to albumin concentration in tested sample . The absorbance was read at 630 nm (Bilabo SA, Company)

10-Estimation of serum globulin:-

To measure of globulin , the levels of albumin were sub-minus from the concentration of total serum protein .

11-Statistical analysis:-

All results of the present study were shown as means \pm stander deviation (SD). SPSS system was used to analysis the data and students t- test was used to explain the differences between control and tested groups at $p < 0.05$ as a lowest significant limit [7]

12-Results

13-C-reactive protein (CRP) and interleukin-6 (IL-6):-

The values of CRP and IL-6 in burned patients and control groups were also illustrated in the table 1- A, B. These results indicated a remarkable increase ($p < 0.05$) in burned patients except concentrations of IL-6 in burned males were appeared non-significantly ($p > 0.05$) compared to control subjects .

14-Total serum protein, albumin, and globulin:-

Results of total serum proteins , albumin , and globulin in males and females affected with second and third degree of burn were shown in table 1- A,B. They were appeared significantly fall ($p < 0.05$) in most burned groups in a comparison with control groups.

-Results are means \pm SD.

-Means having mark * are significantly different at level $p < 0.0$

-Results are means \pm SD

-Means having mark * are significantly different at level $p < 0.05$

Table(1-A):- Shows means of C-reactive protein (CRP ug/ml) , interluleukin-6(IL-6 pg/ml) , total serum proteins(TSP g/dl) , albumin(g/dl),and globulin(g/dl) of healthy and burned male patient groups affected with second and third degree of burn.

| Groups parameters | Healthy | (First group) | (Second group) |
|----------------------|-----------------------|-----------------------|------------------------|
| | control group | Second burn degree | Third burn degree |
| CRP(ug/ ml) | 1.16 \pm 1.158 | 16.02* \pm 1.967 | 17.49* \pm 1.835 |
| IL-6(pg/ml) | 1925.94 \pm 2465.01 | 1734.2 \pm 1104.95 | 3409.97 \pm 2516.165 |
| TSP(g/dl) | 7.54 \pm 1.354 | 4.73* \pm 10129 | 5.13* \pm 0.479 |
| Albumin(g/dl) | 4.80 \pm 0.650 | 3.20* \pm 0.681 | 4.42 \pm 0.484 |
| Globulin(g/dl) | 3.43 \pm 1.221 | 1.53* \pm 1.199 | 0.70* \pm 0.236 |

Table(1-B):- Shows means of C-reactive protein (CRP ug /ml), interleukin-6(IL-6 pg/ml), total serum proteins(TSP g/dl), albumin(g/dl), and globulin(g/dl) of healthy and burned female patient groups affected with second and third degree of burn.

| parameters \ Groups | Healthy | (First group) | (Second group) |
|---------------------|----------------|--------------------|-------------------|
| | control group | Second burn degree | Third burn degree |
| CRP(ug/ ml) | 1.34±1.495 | 16.29*±1.874 | 17.1*±0.892 |
| IL-6(pg/ml) | 1195.7±601.662 | 2474.82*±848.87 | 3804.26*±2147.19 |
| TSP(g/dl) | 7.59±0.766 | 5.32*±1.326 | 5.02*±0.582 |
| Albumin(g/dl) | 4.61±0.210 | 3.61*±0.891 | 4.26±0.409 |
| Globulin(g/dl) | 3.14±1.153 | 1.65*±1.132 | 0.83*±0.303 |

15-Total white blood cells count (WBCs):-

The results of total WBCs which are explained in table 2 -A,B revealed a significant increase ($p < 0.05$) of male burned patients in both groups (second and third degree of burns) in comparison with healthy male group. Results of total WBCs showed a significant elevation ($p < 0.05$) in female burned patients affected with second and third degree of burns when compared with healthy control females.

16-Differential count of white blood cells

17-A granular WBCs

18-Percentage of lymphocytes:-

Concerning lymphocytes percentage which are described in table 2-A,B indicated a significant lowering ($p < 0.05$) in both groups of males suffering from second and third degree of burn in a comparison with healthy male group . Females burned patient groups showed a significant fall ($p < 0.05$) of lymphocytes in both groups when compared with healthy females .

19-Percentage of Monocytes:-

Results of monocytes (table 2-A,B) showed an insignificant drop ($p > 0.05$) of male burned patients of the first group affected with second degree of burns whereas the second group of burned males explained a significant increase ($p < 0.05$) of monocytes percentage when compared to healthy male group .The results were insignificantly elevated ($p > 0.05$) of monocytes in female burned patients of both groups (second and third degree of burns) in comparison with healthy female group .

Table(2-A):-Shows means of total white blood cells (WBCs $10^3/\text{mm}^3$), lymphocytes%, and monocytes% of healthy and burned male patient groups affected with second and third degree of burn.

| Groups parameters | Healthy control group | (First group) Second burn degree | (Second group) Third burn degree |
|-------------------------|-----------------------------|--|--|
| WBCs $10^3/\text{mm}^3$ | 7.32±1.209 | 15.59*±8.253 | 17.68*±7.523 |
| lymphocytes % | 32.12±6.178 | 10.49*±4.103 | 10.77*±5.067 |
| Monocytes % | 8.43±1.915 | 7.95*±3.154 | 12.72*±5.492 |

-Results are means \pm SD.

-Means having mark * are significantly different at $p < 0.05$.

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-Means having mark * are significantly different at $p < 0.05$.

Table(2-B):- Shows means of total white blood cells (WBCs $10^3/\text{mm}^3$), lymphocytes%, and monocytes% of healthy and burned female patient groups affected with second and third degree burns.

| Groups parameters | Healthy control group | (First group) Second burn degree | (Second group) Third burn degree |
|-------------------------|-----------------------------|--|--|
| WBCs $10^3/\text{mm}^3$ | 7.059±1.556 | 16.76*±5.643 | 22.98*±11.733 |
| lymphocytes % | 33.60±8.446 | 13.55*±6.947 | 8.54*±5.311 |
| Monocytes% | 7.204±1.851 | 10.49±5.563 | 8.28±3.863 |

20-Granular WBCs

Percentage of Neutrophils:-

The results of neutrophils that are shown in table 3-A,B have been explained a significant elevation ($p<0.05$) in males burned patients of both groups (second and third degree of burns) when compared to healthy males group . Also, the results of burned females showed a significant heighten ($p<0.05$) of neutrophils of both groups (second and third burn degree) in comparison with healthy female groups .

21-Percentage of Eosinophils:-

The results which are presented in table 3-A,B show an insignificant lowering ($p>0.05$) of eosinophils percentage in male burned patients of second degree of burns (First group) and a significant decrease ($p<0.05$) of eosinophils in male burned patients of second group affected with third degree of burns when compared to healthy male group . Levels of eosinophils in female burned patients also showed a significant fall ($p<0.05$) in both groups (second and third degree of burns) in comparison with healthy control females .

22-Percentage of Basophils:-

Results which are explained in table 3-A ,B showed a significant drop ($p<0.05$) in basophils of male burned patients affected with second degree of burns .On the other hand, there is an insignificant decline ($p>0.05$) of basophils level in males burned patients affected with third degree of burns when compared with healthy males group . Data obtained from burned females indicated a significant lowering ($p<0.05$) of basophils in females burned patients affected with second degree of burns . Levels of basophils in burned females of third degree showed an insignificant decrease ($p>0.05$) when matching with healthy control females group .

Table(3-A):- Shows means of neutrophils%, eosinophil's%, and basophils% of healthy and burned male groups affected with second and third degree of burns.

| Groups parameters | Healthy control group | (First group) Second burn degree | (Second group) Third burn degree |
|----------------------|-----------------------------|--|--|
| Neutrophils % | 55.96±7.562 | 79.74*±6.016 | 75.43*±9.646 |
| Eosinophil's % | 2.547±1.115 | 1.374±2.139 | 0.228*±0.454 |
| Basophils % | 0.903±0.171 | 0.486*±0.207 | 0.937±0.755 |

-Results are means ±SD.

-Means having mark * are significantly different at $p < 0.05$.

Table(3-B):- Shows means of neutrophils %, and basophils% of healthy and burned males patients suffering from second and third degree of burns.

| Groups parameters | Healthy control group | (First group) Second burn degree | (Second group) Third burn degree |
|----------------------|-----------------------------|--|--|
| Neutrophils % | 58.49±10.632 | 75.87*±12.431 | 82.38*±9.429 |
| Eosinophil's % | 2.299±1.506 | 0.179*±0.242 | 0.166*±0.235 |
| Basophils % | 1.051±0.517 | 0.628*±0.331 | 0.626±0.463 |

-Results are means ±SD.

-Means having * are significantly different at $p < 0.05$.

23-Discussion

C-reactive protein :-

According to principal facts indicate that CRP is an acute phase protein and it tends to increase during burn injuries. CRP is a circulating biomarker of inflammation, it synthesized by hepatocytes as a response to higher levels of cytokines(IL-6 and IL-1B) [8]. It is well documented that inflammation , infection or sepsis can increase response of the body to produce many inflammatory cytokines especially CRP in burned patients [9]. [10] found that CRP is increased at post burn and tend to decrease during hospitalization . Also , they have been indicated that increase area of burn injury is proportional with a high levels of CPR and these levels are more in females than of males ,and the survival rates of burned patients is increase as the levels of CRP become decreased during hospital period [11] [12] [13]. Study of Piroglu *et al.*,(2016) showed that the levels of CRP were not significantly increased during first hours of burn and found that procalcitonin levels were increased

24-Interleukin-6 :-

It is well observed that IL-6 is more increased in burned patients and these observations are consistent with studies of [14] and [15] which indicated an increased levels of Il-6 at first day after burn and progressively increased gradually until reach to its peak at six day of burn and may be continued with elevation at 21 of burn.Study of [16] proved a relationship between IL-6 and many cutaneous inflammatory diseases. Sex hormones exert an excitatory role to induce immune system, particularly, in females . So , in females , the immune responses are more active than of males [17] [18]. Females appear to have a high levels of IL-6 and these observations can be illustrated that females have immune response more active than of males , the immune response in females is confirmed through experimental studies of mice that performed on post- burn therapy with an anti- IL-6 neutralizing antibodies lead to partially back the delayed type

of hypersensitivity, also, there is a suppression of immune response is found at 10 days of post – burn mediated by increased levels of IL-6 [19]. The results of the present study are supported by other documents, since, it has been found that sexual hormone, in particular, estradiol (E2) can be to modulate immune response in females after exposure to traumatic events [20]. In addition, previous study found gender variation in estradiol concentration in patients affected with septicemia, since, E2 is found to elevate three to five times in affected males more than of normal males and its levels can be restored to normal values after five days, inversely, the level of E2 concentrations ranged between 10 -15 fold in burned females and continue for 7-8 days [21]. E2 exerts an inhibitory factor to down-regulate releasing of IL-6 from many cells including bone generating cells (osteoblasts) and macrophages [22] [23]. On the other hand, study of [24] indicated that in postmenopausal women, there is an increased levels of many cytokines, particularly IL-6 when these women are subjected to stress conditions.

25-Total serum proteins, albumin and globulin:-

The results obtained from this study indicated overall lowering of serum protein components in all burned patient groups, these data are consistent with previous researches that confirmed a decrease of proteins in blood in burned patients and showed that hypoalbuminemia is prominent feature particularly in burn and associated many complications such as increase extravascular fluids, edema, poor healing of wound, and susceptibility to infection [25] [26]. As the burn injuries are associated with drop of globulin production, the release of immunoglobulin G in response to T-cell dependent antigens become depressed because of severity of burn [27]. Another study indicated different results and confirmed that there is a compensatory up-regulation of plasma globulin Sheridan [28]. The common explanation of these data is dependent on the previous studies that indicated, in normal body states, liver is engaged in the synthesis and release of essential constitutive proteins particularly albumin and prealbumin, and this function tend to be disturbed at incidence of trauma and liver functions become shifted toward synthesis of acute phase proteins such as CRP, the change of liver function is named acute phase response and occur to improve homeostatic mechanisms of the body [29] [30]. Burned skin seems to permit the secretion of different vasoactive molecules causing increase permeability of water, albumin, and other large protein molecules diffuse across blood vessels [31]. In previous report, it is observed that the peak of response to inflammatory markers reach to its peak at 12- 24 hours of burn and associated with increase synthesis of acute phase reactants, on the other hand, albumin mRNA become inhibited causing hypoalbuminemia [32]. Burned subjects are exposed to increase hypermetabolic state causing mobilization of protein and other nutrient molecules to release of energy, the catabolism of protein seems the prominent process causing a negative nitrogen balance.

26-Total white blood cells (WBCs):-

As for the results of white blood cells have been found significantly increased ($p < 0.05$) of WBCs in men and women complained from second and third degree of burns. The recent data are consistent with study of [31] that confirmed a substantial increase in total leukocytes in burned patients, but other previous study showed conflicted data through down regulation of leukocytes in burn conditions [6].

Previous reports in state of burn and blunt trauma, the WBCs are mediated in progression of organ failure far damage of burn Earlier [33] [3]. So, the incidence of respiratory dysfunction occurs after burn injury is resulted from increase amount of leucocytes in circulation [34]. It is well observed increase WBCs happen rapidly after burn wound and then after back or decrease to normal and remain up to week [3] [35]. Studies are conducted on burned animals showed that many organs such as liver, skin, and pulmonary tissues become infiltrated with polymorphonuclears [36] [37].

It could be believed that increased of leukocytes happen as a consequence of production of many inflammatory mediators and other cytokines that in turn increase of more WBCs into blood circulation to impair infections and increase defense against burn associated infection.

27-A granular white blood cells

Lymphocytes:-

The results of lymphocytes percentage appeared markedly lowered ($p < 0.05$) in all burned groups of affected with second and third degree of burn. These data are consistent with study of [11] which observed decrease of lymphocytes in burned subjects and it attributed this depletion to increase margination and extravastation of lymphocytes. Also, it is documented that IL-6 is implicated in the suppression of lymphocytes in burn conditions because of inhibitory effect of IL-6 on function of T-cells [38].

28-Monocytes:-

There is insignificant drop ($p > 0.05$) of monocytes percentage in male burned patients suffering from second degree of burn, whereas third degree of burn explained a remarkable elevation ($p < 0.05$). Female burned patients explained an insignificant increase of monocytes in both degree of burns.

On the basis of pathological and physiological point view, the monocytes appear respond quickly to release of inflammatory cytokines including CRP and IL-6 in circulation and migrated readily to affected area of burn, more ever, during burn, there is accelerated permeability of blood vessels in damaged area to enable of monocytes to mature in to competent cells, these change may be play role rendering number of monocytes remains at border line and do not exceed to peak.

29-Granular white blood cells

Neutrophils:-

The level of neutrophils indicated elevation ($p < 0.05$) in both sexes of burned patients with second and third degree of burns. The present results are inconsistent with the previous studies [39] and [40] that confirmed a drop of neutrophils in thermal injuries.

It is previously confirmed that increase morbidity and mortality in burned patients is return to infection which presented the disastrous problem during thermal burn . Neutrophils play first line of defense against infection after injuries and the infection the major threats to burned subjects and his survival [41] [42]. Other reports document that a diminished oxidase activity in PMNs from burn patients and this may take part to the increased hazard of infection by affecting the oxygen-dependent activity of PMN bactericidal activity [43] [44]. Acute inflammatory reactions lead to an increase of neutrophil margination and adherence to endothelium of blood vessels .

The localized neutrophil at inflamed area can be propagated damage of vascular walls and permit to produce of their granule components causing edematous and hemorrhagic areas [45] [46].

Results of this study can be supported on the basis of the fact confirmed that some components presented in neutrophils called heat shock protein (HSPs) exert defense against environmental fluctuations and stresses. Burn act to increase and activate appearance HSPs in neutrophils that accompanied with an increase of oxidative stress and apoptosis . Expression HSPs may be maintained and minimized of response to oxidative stress that may increase life time of neutrophils in patients – [42].

30-Eosinophils:-

Eosinophils percentage revealed an insignificant fall ($p > 0.05$) in male burned patients affected with second degree of burns, whereas in third degree showed a significant decrease ($p < 0.05$). Similarly, burned females explained a significant drop ($p < 0.05$) affected with second and third degree of burn.

Production of eosinophils undergoes from specific phenomenon called diurnal fluctuation and this results because of glucocorticoid hormone secretions at night make the number of eosinophils at a higher levels than of other [47]. It is well noted increase release of cortisol, a potent glucocorticoid hormone, and catecholamines in burn injury [48] rendering the production of eosinophils suppressed . The effective therapeutic options to treat many of inflammatory states in particular severe acute inflammations, and glucocorticoids exert many effects on granulocyte apoptosis [49] and phagocytic activity of macrophages [50]. Experimental study of [51]. supported the present data and confirmed present of glucocorticoid receptors on eosinophils may increase apoptosis rate of eosinophils and inversely delay of neutrophil apoptosis in vitro cultures.

31-Basophils:-

The levels of basophils did show a marked drop at $p < 0.05$ in burned males affected with second degree, and inremarkable increased at $p > 0.05$ in third degree of burned males. In females, basophils pointed out a significant decrease ($p < 0.05$) in patients with second degree and insignificant decrease ($p > 0.05$) in burned females of third degree . the studies that involved basophils in burned patients appear not enough.

One possible attribution to explain the fluctuations and changes occurring in values of basophils noted in both degree of burn can be return to many processes including interaction among many hemostatic mechanism , inflammatory mediators , cytokines production, migration of WBCs, and apoptic factors may be implicated in this variation .

32-Conclusions

According to data obtained from this study and explained above , one can be concluded that the patients of burn have elevated levels of inflammatory mediators in particular IL-6 and acute phase proteins such as CRP which in turn increase apoptosis levels of leukocytes and lead to increase susceptibility of patients to infection . In addition , stress of burn is accompanied with increase production of glucocorticoids and catabolism of proteins can be to inhibit immune responses in burned patients . It is well noted that females have much levels of IL-6 than of males that make the burned females more susceptible to infection and render the health of burned females become worse and aggravated higher than of males .

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