Acute Lung Injury following Hemorrhagic Shock is governed by macrophage related factors that acts through neutrophils infiltration in a Hemorrhagic Shock rat model

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الضرر الرئوي الحاد نتيجة صدمة كلومية يحدد من قبل عوامل تتعلق بالخلايا البالعة الكبرى والتي تؤدي الى ترشيح الخلايا البالعة المتعادلة لدى نموذج فئران مصابة بصدمة كلومية

الخلاصة

المقدمة:تتميز متلازمة الاعتلال الرئوي الحاد في الصدمة الكلومية بضرر رئوي حاد مع معدل وفيات عالي ومع ذلك لاتزال آليته غير مفهومة. الصدمة الكلومية ومايتبعها من انتعاش يؤدي الى متلازمة استجابة التهابية جهازية والتي بدورها تفضي الى ضرر رئوي حاد بالاضافة الى اختلال وظيفي في الاعضاء الاخرى ، هذه الدراسة تهدف الى تقييم التاثير الوقائي المحتمل لنقص الخلايا البالعة الكبيرة في الفئران المصابة بضرر رئوي حاد نتيجة الصدمة الكلومية.

الطريقة:تم تقسيم ٢٨فارا بالغا امهق اللون الى اربع مجاميع كل مجموعة احتوت على سبعة فئران وهي مجموعة الزيف ومجموعة الضبط غير المعالجة ومجموعة الفئران المعالجة بمادة مثبطة ومجموعة الضبط مع وسيلة النقل فقط بدون مادة مثبطة . لقد تم تعريض الفئران الى صدمة كلومية لمدة ساعة واحدة ومن ثم تم انعاشها بمحلول ملحي نوع (رنكر) لمدة ساعة واحدة. استنفاد الخلايا البالعة الكبيرة تم باستخدام مادة كلودرونيت ثنائي الصوديوم والمحمولة عن طريق الليبوسوم كوسيلة نقل المادة تم اعطائها وريديا بجرعة ١٠ وحدات لكل ١٠غم من وزن الفار وهذا المحلول اعطي بشكل جرعتين بينهما يومان كاملان وتمت الجراحة بعد مرور يومين من اخر جرعة. المجموعة الاخيرة اعطيت فقط الليبوسوم بدون المادة المثبطلة. تم استئصال الرئتان جراحيا وثبتتا في محلول١٠% فورمالين لغرض الفحص النسيجي والتلف الرئوي تم تقييمه باستخدام الفحص النسيجي المرضي. الخلايا البالعة المتعادلة تم صبغها باستخدام تقنية المناعة اللاصقة او الفلورية.

النتائج :- هذه الدراسة بينت وجود فرق احصائي هام بين مجموعة الزيف ومجموعة الضبط غير المعالجة ($P<0.05$) كما ان ١٠٠% من مجموعة الزيف كانت لديها اصابة رئوية طبيعية بينما ٧١% من مجموعة الضبط غير المعالجة وجدت لديها اصابة رئوية شديدة في حين ٨٥% من مجموعة الفئران المعالجة بمادة مثبطة كانت لديها فقط اصابة رئوية بسيطة كانت هنالك زيادة في انعزال الخلايا البالعة المتعادلة في الفئران التي تعرضت لصدمة كلومية في حين انها انخفضت في الفئران المعالجة بمادة مثبطة . الفئران ذات الصدمة الكلومية وجد لديها زيادة بعشرة اضعاف في عدد الخلايا البالعة المتعادلة المتجددة بالمقارنة مع مجموعة الزيف($p<0.05$) الفئران التي اعطيت كلودورنيت كان لديها انخفاض في الخلايا المتجددة بالمقارنة مع مجموعة الضبط ($p<0.05$).

الاستنتاج: الدراسة الحالية ترفع فهمنا الى مستوى جديد حيث انها تظهر بوضوح الدور الاساسي للخلايا البالعة الكبرى كوسيط اولي في افراز مواد تؤدي الى تجديد الخلايا البالعة المتعادلة في الرئة خلال الصدمة الكلومية هذه الاخيرة تمارس دورها الضار من خلال افراز اللوكوترايين.

Abstract

Background:- Adult respiratory distress syndrome in hemorrhagic shock is characterized by acute lung injury with a high mortality rate and yet its mechanism is poorly understood. Hemorrhagic shock followed by resuscitation induces a systemic inflammatory response syndrome that results in acute lung injury and other organ dysfunction. This study was designed to assess the possible protective effect of macrophage depletion in hemorrhagic shock-induced acute lung injury.

Methods:- 28 adult Albino rats were divided into four groups each containing seven rats: sham group, control group, macrophage depleted group and their vehicle control group. Rats underwent hemorrhagic shock (HS) for 1hr then resuscitated with Ringer's lactate (1hr) (induced untreated group, HS); The macrophages depletion is done using clodronate disodium carried on liposome as a vehicle, the drug is administered intravenously in dose of 10 units for each 10 g of rat weight, this macrophage depleting agent is given in two doses two days apart and the surgery is done after two days from the second dose, the liposome is administered alone. The lungs were harvested, excised and was fixed in 10% formalin for histological examination. Lung injury was assessed histopathological and neutrophil was stained using immunoflurouscent technique.

Results:- There was statistically significant difference between induced untreated (HS) group and sham group ($P< 0.05$). 100% of the sham group had normal lung injury while 71% of the control group had severe lung injury d up to 85% of macrophage depleted group has only mild lung injury**.** There is an increase in PMN sequestration in the shock animals and a decrease in the macrophage depleted groups. Shock rat had a 10-fold increase in PMN recruitment vs. sham animals, $p<0.05$. Rat receiving clodronate had a significant decrease in recruitment vs. control $p<0.05$.

Conclusions:- The current study advances our understanding another level by demonstrating the essential role of the macrophage as initial mediator in secretion an elements that recruits neutrophils in lung during hemorrhagic shock, that will exert their injurious effect through leukotrienes.

Key Words: macrophage, neutrophil, hemorrhagic shock, acute lung injury

Background:-

Adult respiratory distress syndrome in hemorrhagic shock is characterized by acute lung injury with a high mortality rate and yet its mechanism is poorly understood. Recent studies have demonstrated a significant role for factor(s) present in mesenteric lymph following hemorrhagic shock in the etiology of post-hemorrhagic shock acute lung injury (ALI). Earlier studies have shown that ischemia-reperfusion insults to systemic tissue beds can also result in ALI. Factors in systemic lymph may cause lung injury after hemorrhagic shock.[(1)]

Mesenteric lymph is the mechanistic link between gut ischemia/reperfusion (I/R) and acute lung injury (ALI) following hemorrhagic shock (HS). [(2)] ALI is the result of an inflammatory process involving neutrophil (PMN) recruitment/priming/activation.[(3)] Leukotrienes are inflammatory mediators derived from the metabolism of AA by 5-lipoxygenase (5-LO) and its coenzyme 5-LO activating protein. [(4)] LTB4 is a leukotriene that stimulates neutrophil (PMN) chemotaxis, increases PMN adherence to endothelial cells, stimulates the release and generation of superoxide radicals, and can even increase 5-LO activation in PMNs to produce more LTB4.[(5)] LTC4 is another

leukotriene that provokes lung edema formation and bronchial constriction. Elevated levels of leukotrienes are seen in several inflammatory diseases such as psoriasis, inflammatory bowel disease, and acute respiratory distress syndrome.[4] Prior studies have shown that hemorrhage (Hem) can serve as a priming stimulus for acute lung injury (ALI) triggered by subsequent septic challenge (cecal ligation and puncture, CLP). Furthermore, in vivo antibody neutralization of the chemokines, macrophage inflammatory chemokine-2 (MIP-2) and keratinocyte-derived chemokine (KC), immediately after hemorrhagic shock appears to differentially affect the onset of ALI. This is due to divergent effects of MIP-2 and KC on Hem-induced neutrophil (PMN) priming.[6] We hypothesize that macrophages governed the PMNS sequestration into lung parenchyma and it is critical for the development of ALI through leukotrienes following HS.

Methods:-

1. Animals protocol

A total of twenty eight adult male Albino rats weighing 150-200 g were purchased from Animal Resource Center, the Institute of embryo research and treatment of infertility, Al-Nahrain University. They were housed in the animal house of Kufa College of Medicine in a temperature-controlled (25°C) room with alternating 12-h light/12-h dark cycles and were allowed free access to water and chow diet until the start of experiments. After the 1st week of acclimatization the rats were randomized into three groups as follow:

Sham group: this group consisted of 7 rats; rats underwent the same anesthetic and surgical procedures for an identical period of time as shock animals, but neither hemorrhage nor fluid resuscitation was performed.

Control group: (induced untreated group): this group consisted of seven rats; rats underwent hemorrhagic shock (for 1hr) then resuscitated with Ringer's lactate (RL) (for 1hr), and left until the end of the experiment.

Macrophages depleted animals and their control group. (7 animals each group) The macrophages depletion is done using clodronate disodium carried on liposome as a vehicle, the drug is administered intravenously in dose of 10 units for each 10 g of rat weight, this macrophage depleting agent is given in two doses two days apart and the surgery is done after two days from the second dose, the liposome is administered alone for the control group

2. Hemorrhagic Shock Protocol

Animals were intraperitoneally anesthetized with 80 mg/kg ketamine and 8 mg/kg xylazine and subjected to a 50% blood loss (30 ml/kg) via intracardiac puncture from the left side of the chest over 2 min and left in shock state for 1hr. The animals were then resuscitated with two times blood loss (60 ml/kg) using intravenous lactated Ringers via tail over 1 hr. The sham group underwent all instrumentation procedures, but neither hemorrhage nor resuscitation was carried out. Animals were allowed to breathe spontaneously throughout the experiment. Two hour after the completion of resuscitation, rats were again anesthetized and sacrificed by exsanguinations, where the chest cavity was opened and blood samples were taken directly from the heart. The lungs were harvested, and fixed in 10% formalin for histological examination.

3. Tissue Sampling for Histopathology

At the end of the experiment, rats were sacrificed and the lung was harvested. All specimens were immediately fixed in 10% buffered formalin. After fixation they were

processed in usual manner. The sections were examined by microscope then the histological changes were determined.

The degree of lung injury was assessed using the scoring system described by **Matute-Bello *et al.* (2001)** that graded congestion of alveolar septae, intra-alveolar cell infiltrates, and alveolar hemorrhage.[7] Each parameter was graded on a scale of 0–3, as follows: alveolar septae, 0: septae thin and delicate, 1: congested alveolar septae in <1/3 of the field, 2: congested alveolar septae in 1/3–2/3 of the field, 3: congested alveolar septae in >2/3 of the field; intra-alveolar cell infiltrates, 0: <5 intra-alveolar cells per field, 1: 5 to 10 intra-alveolar cells per field, 2: 10 to 20 intra-alveolar cells per field, 3: >20 intra-alveolar cells per field; Alveolar hemorrhage, 0: no hemorrhage, 1: at least 5 erythrocytes per alveolus in 1 to 5 alveoli, 2: at least 5 erythrocytes in 5 to 10 alveoli, 3: at least 5 erythrocytes in >10 alveoli. The total lung injury score was calculated be adding the individual scores for each category and lung injury was categorized according to the sum of the score to normal (0), mild (1-3), moderate (4-6) and severe injury (7-9). The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals, figure (1).

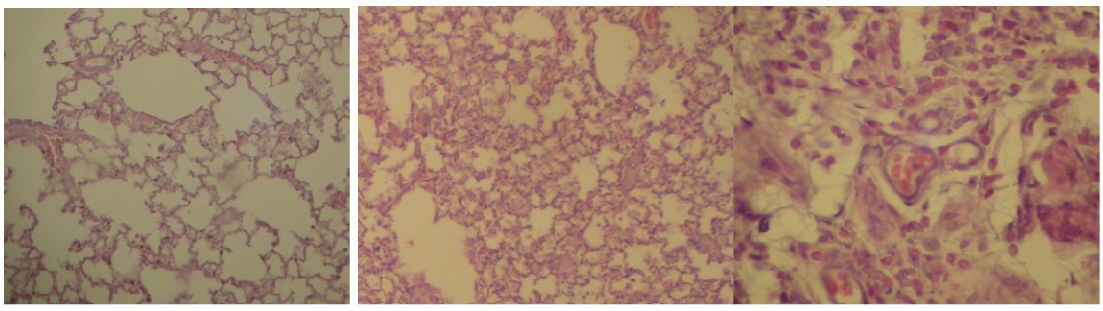
4. immunofluorescent (IF) staining

Lung sections that underwent immunofluorescent (IF) staining against rat PMNs. Red indicates PMNs, blue indicates cell nuclei, and green indicates cell membranes(this is done in collaboration with cardiothoracic research center, Aurora, Colorado, USA)

Results:-

1. Histological finding

A cross section of sham rat's lung showed the normal appearance of all three parameters (thin and delicate alveolar septae, no intra-alveolar cell infiltrates and no alveolar hemorrhage). There was statistically significant difference between induced untreated (HS) group and sham group ($P < 0.05$). 100% of the sham group had normal lung injury while 71% of the control group had severe lung injury d up to 85% of macrophage depleted group has only mild lung injury as shown in figure (2)



mild moderate severe

Figure (1): Photomicrograph of lung sections with different histological findings. The section stained with Haematoxylin and Eosin.

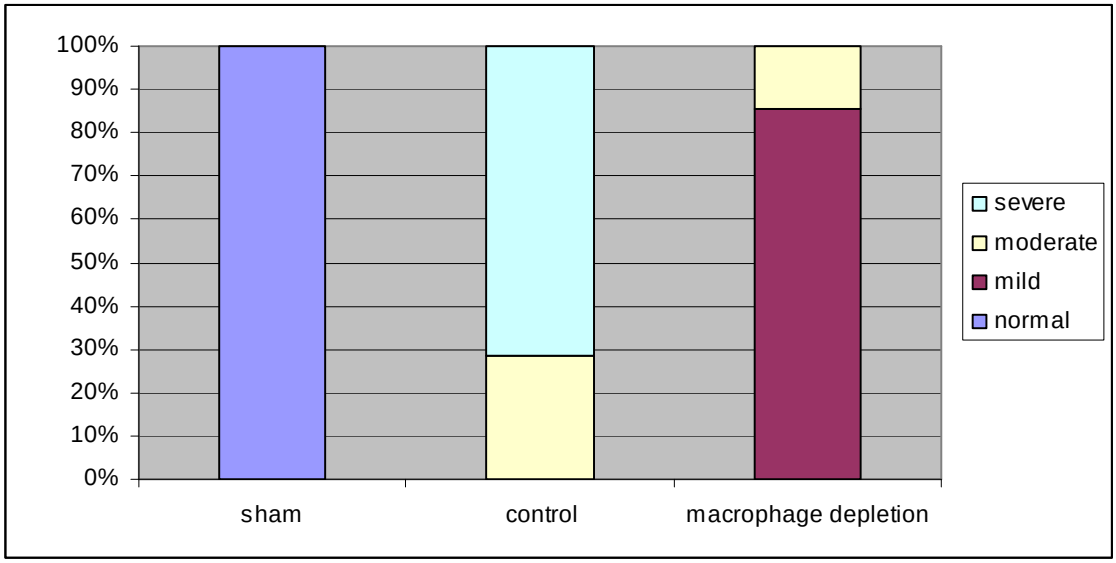
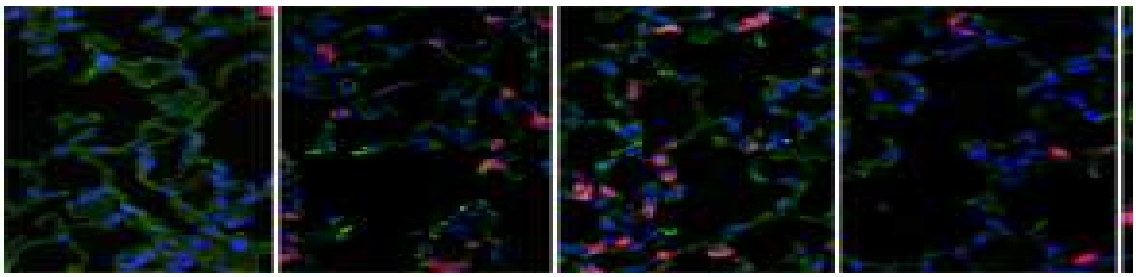


Figure (2) The differences in histopathological grading of abnormal lung changes among the three ex perimental groups.

2- neutrophils sequestration : as shown in The pictures below are representative lung sections that underwent immunofluorescent (IF) staining against mouse PMNs Red indicates PMNs, blue indicates cell nuclei, and green indicates cell membranes. There is an increase in PMN sequestration in the shock animals and a decrease in the macrophage depleted groups figure (3). Shock rat had a 10-fold increase in PMN recruitment vs. sham animals, $p<0.05$. Rat receiving clodronate had a significant decrease in recruitment vs. control $p<0.05$. figure (4)



sham shock shock + vehicle shock + macrophage depletion

Figure (3): lung sections that underwent immunofluorescent (IF) staining against mouse PMNs.

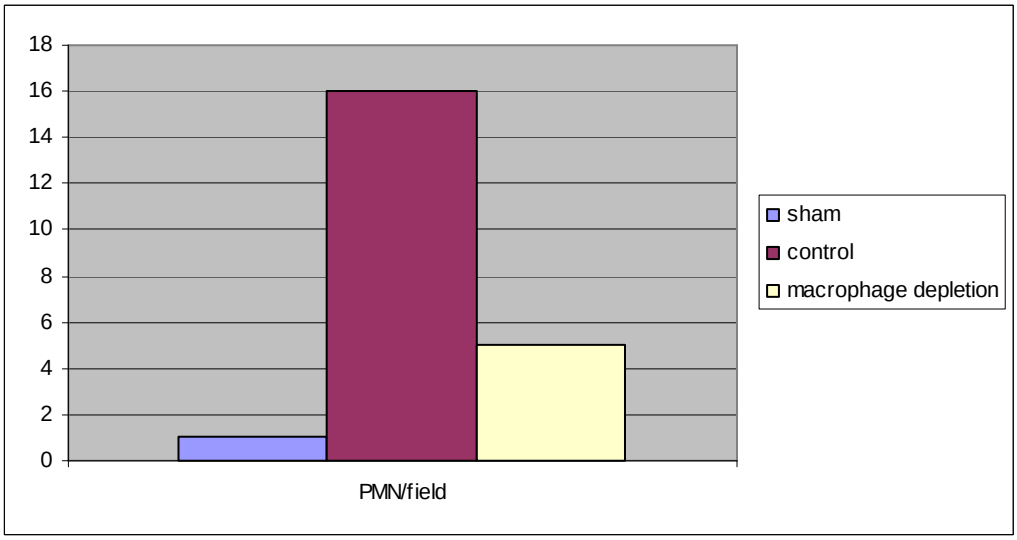


Figure (4): Shock rat had a 10-fold increase in PMN recruitment vs. sham animals, $p<0.05$. Rat receiving clodronate had a significant decrease in recruitment vs. control $p<0.05$. The average area of the lung field was 4220 microns.

Discussion:-

Lung injury is a hazardous sequel of hemorrhagic shock and research work on this complication will build up the therapeutic strategy in treatment the hemorrhagic shock associated lung injury. In this research we demonstrated that hemorrhagic shock causes acute lung injury which is reflected by histopathological changes. The severity of lung injury is strongly associated with increase in the neutrophils sequestration and in turn we can conclude that neutrophils infiltration is a marker of severity of lung injury in hemorrhagic shock. In previous studies declared that leukocytes accumulated in the lungs as observed in the histological section of the shocked rat lung where activated neutrophils following hemorrhagic shock are capable of releasing cytotoxic products including leukotrienes, and the intrinsic 5-lipoxygenase activity is required for neutrophil adherence and chemotaxis and neutrophil-mediated lung injury. [3,6]. In addition to neutrophils, alveolar macrophages and circulating macrophages aggravate lung injury and alveolar neutrophil sequestration in hemorrhagic shock [3,7] and might contribute to further release of leukotrienes. In this study we have demonstrated that macrophage is an important element in the acute lung injury following hemorrhagic shock and afctors released from macrophage may induce neutrophils sequestration and further release of leukotrienes.

Conclusions:- The current study advances our understanding another level by demonstrating the essential role of the macrophage as initial mediator in secretion an elements that recruits neutrophils in lung during hemorrhagic shock, that will exert their injurious effect through leukotrienes. These findings are promising for the eventual therapeutic role of specific macrophage or neutrophil inhibitors in the prevention of post shock lung injury and subsequent multiorgan failure.

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