Interplay of Serum Procalcitonin and Renal Tissue Caspase 3 as Diagnostic Markers of Sepsis in Lipopolysaccharide Rat Model of Sepsis

Nessren M. Abd el-Rady

Department of Medical physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

Background: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and its rapid, accurate diagnosis can cause a huge burden to whole families and to society in terms of medical costs. Renal failure induced by sepsis is still an exasperating problem in the clinical inquiry. **Aim**: This paper examines a new biomarker; serum procalcitonin (PCT) in the diagnosis of sepsis based on intraperitoneal injection of lipopolysaccharide (LPS). PCT is a a calcitonin precursor secreted mainly from the liver. Whether PCT shows efficacy in improving diagnosis of sepsis and the accompanying renal cell dysfunction by the expected successive apoptosis after sepsis were explored in this paper. **Methods:** 40 adult male albino rats were randomly equally divided into 2 main groups: The control group: received intraperitoneal saline (IP) and LPS injected group: received 10 mg/kg of lipopolysaccharides (LPS), from *Escherichia coli* IP once. The rats were monitored using a mouse clinical assessment score for sepsis (M-CASS) for 48 hours. Body temperature, Systolic and diastolic blood pressure and serum glucose level, leucocytic count , serum creatinine and urea and serum procalcitonin were estimated . Immunohistochemical staining of caspase 3-cellular expression in renal tissue. **Results:** increase mortility rate in septic rats. Serum PCT was significantly increased in septic rats with high sensitivity and specificity. Significantly increased levels of serum urea and creatinine, reduced levels of blood glucose, and increased renal caspase 3 expression were exhibited in the LPS injected group. Moreover, tachycardia, hypotension and hypothermia were highly significantly increased in the LPS injected group compared to those in the control group. The M-CASS behavioral changes were all detected after LPS injection. **Conclusion**: Serum procalcitonin is a noval, accurate and specific biomarker that shows efficacy in the diagnosis of sepsis and its associated renal dysfunction, which explained by apoptosis and the increase in renal tissue caspase 3 expression.

Nessren M. Abd el-Rady: Department of Medical physiology, Faculty of Medicine, Assiut University, Assiut, Egypt, Email: nessrenmahmoud05@gmail.com; Mob., +201003248010

INTRODUCTION

Sepsis is a life-threatening syndrome with a high mortality and morbidity rate due to organ dysfunction secondary to infection (48). The standard clinical diagnosis of sepsis requires finding a focus of infection that is accompanied by at least 2 signs of the systemic inflammatory response syndrome. These systemic signs; in human; include changes in body temperature (greater than 38 or less than 36°C), heart rate (greater than 90beats/min), respiration (greater than 20 breaths/min), or arterial partial pressure of CO_2 less than 32 mm Hg) and white blood cell counts (greater than $12 \times 10^3/$ mm^3, less than $4 \times 10^3/$mm^3, or greater than 10% bands) (5)**.** But in animals, appear in form of fever, anorexia, weight loss, or other signs of illness (59). It could be complicated by shock, tissue damage, multiple organ dysfunction, and even death (23, 57). The bacterial peritonitis, and peptic ulcer with Helicobacter pylori origin (36), respiratory tract infections, urinary tract contaminations and sepsis are more frequent serious septic conditions (37).

Diagnosis of bacterial diseases is now and then is a serious misleading problem since clinical signs of contaminations from distinctive causative agents can be similar (22). For case, in accord to WHO it may be troublesome to distinguish viral from bacterial diseases in different cases (60). Most common characteristic laboratory tests used for diagnosis of bacterial infection include measuring the number of leukocytes in peripheral blood, differential count of leukocytes, erythrocyte sedimentation rate, C-reactive protein (CRP), tumor necrosis factor alpha, interleukin-1,

interleukin-6, interleukin-8, and complement fragment C3a (15, 27). But all of them are not totally accurate, not particular, and require much time to be predictable.

Procalcitonin (PCT) is a 116 amino acid protein, a calcitonin precursor, secreted from C type cells of thyroid gland (12). The liver may be a key source of PCT. It is also found in multiple organs, as lung, kidney, adrenal tissue, monocytes, granulocytes, testis, prostate organ and small intestine (41, 29). Half-life of procalcitonin in serum is 20-24 hours, which makes it reasonable for day by day checking. (32). Beneath typical conditions, insignificant serum PCT concentrations are ordinarily recognized (4). Procalcitonin level rises in bacterial, parasite and yeast contaminations .Raised procalcitonin levels show up in inflammation of an infectious etiology with systemic signs. In this manner, procalcitonin determination is helpful in the diagnosis of sepsis. And it is imperative in controlling the course of treatment (15).

Sepsis can be rapidly complicated by organ failure mostly starts in the kidney. It mainly occurs due to apoptosis in which deoxyribonucleic acid (DNA) fragmentation and cell death occur as a result of activation of death-inducing receptors or intracellular specific serine proteases (caspases) (24). It is the key manager in pro- and anti-inflammatory processes. It is considered an important cause of immunologic suppression in septic conditions, and improving survival by cutting down the inflammatory response accompanying them(55).

In the current study, we used a rat sepsis model done by intraperitoneal injection of lipopolysaccharides (LPS) from *Escherichia coli*

for this experiment, which induces a clinically relevant polymicrobial sepsis (Ofer *et al*, 2015).We aimed to assess the effects of sepsis on rat behavior (rat fur, activity, face grimacing, occurrence of diarrhea and respiratory distress) ,vital signs (temperature, mean systemic systolic &diastolic blood pressure and heart rate) , laboratory blood tests (WBCs count ,serum urea &creatinine and serum procalcitonin) and immunohistochemical staining of renal tissue to detect caspase 3-cellular expression. And to analyze the serum procalcitonin levels as a specific accurate biomarker for detection of bacterial infection in rat model of sepsis.

Materials and methods
Drugs and chemicals:

Lipopolysaccharides (LPS), from *Escherichia coli,* EH100, were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Serum creatinine, urea and Serum Procalcitonin were measured using their specific ELIZA KITS purchased from SinoGeneClon Biotech Co., Ltd, China. All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

Animals and ethics:

All the procedures used in this study were approved and performed in accordance with the internationally accepted guidelines for the Care and Use of Laboratory Animals and were approved by the ethics committee at the Faculty of Medicine, Assiut University, Assiut, Egypt.

40 adult male Wistar albino rats obtained from (weighing about 250-300 g) obtained from the Animal House of the Faculty of Medicine, Assiut University, Assiut, Egypt. The rats were maintained in a room at 22 ± 1 °C with a 12 h light cycle and 60% humidity and kept in a well-ventilated room for 1 week before the experimental work. They were provided food and tap water at room temperature ad libitum.

Experimental design:

40 adult male albino rats were used in the experiment; and randomly divided into 2 main groups:

Control group: 20 rats were injected intra-peritoneally with 1 ml of normal saline

LPS injected group: 20 rats received single intraperitoneal injection of 10 mg/kg of lipopolysaccharides (LPS), from *Escherichia coli,* EH100 to induce general toxemic condition (31)

In the next 48 hours:

1-We monitored animals using a mouse clinical assessment score for sepsis (M-CASS) (39) (table 1). Animals who were gaining a score of 18 or higher at any time were considered moribund rats and should be immediately scarified by cardiac puncture under anesthesia (20).

Table (1) showing mouse clinical assessment score for sepsis (M-CASS)

	Score 1	Score2	Score 3	Score 4
Fur aspect	Actively grooming	Dulling of hair coat	Rough hair coat	Piloerection
Activity	Normal	Reduced	disturbed little	Nil
Behavior	Normal, no abdominal splinting	Slightly hunched, moving freely, mild splinting	Hunched with stiff movement/ posture, moderate splinting	Hunched with, severe splinting
Face	Normal	Normal	moderate grimacing	severe obvious grimacing
Diarrhea	None	Mild	Moderate	Severe
Respiratory distress (dyspnea)	none	Mild	Moderate	Severe

2- *Rat body temperature* is measured rectaly using mercury thermometer

3- *Systolic and diastolic blood pressure* were measured in conscious rats before the start and at end of the experiment by the indirect tail cuff method using Electrosphygmomanometer Pneumatic Pulse Transducer (Model LE 5001 Pressure Meter, Panlab, Harvard, USA). The animals were pre-warmed in a metal chamber at about 35 °C and allowed to acclimate for 30 min before the recordings were made. Three successive readings were made at the same time of day (8-10 a.m.). Mean values from the three measurements was calculated.

4-*Overnight-fasted serum glucose levels* were determined by measuring tail vein blood glucose level using a FIABiomed blood glucose-meter (Germany) at the beginning and before scarfing.

5-*Blood samples* were taken from the retro-orbital vein, centrifuged at 3000 round per minute (rpm) for 15 minutes and the clear, non haemolysed supernatant sera were removed and kept at -20 C until use.

White blood count was obtained using a Coulter counter (Hemavet 950FS; Drew Scientific Inc., Oxford, CT, USA). Leukocyte differential was confirmed with blood smears and manual counting.

Serum creatinine and urea were measured spectrophotometrically using a kit from Biolabo (REF80107, Maizy, France) following the kit instructions.

Serum Procalcitonin was measured using Procalcitonin (pct) ELIZA kit according to its data sheet instruction.

Then after 48 hours, all animals were sacrificed by cervical dislocation under light phenobarbital anesthesia.

5- Immunohistochemical staining of caspase 3-cellular expression in renal tissue.

Caspase-3 (diluted 1:50) was used for detection of apoptosis in kidney cells. Kidneys were taken from all animals, fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. 5 μm thick paraffin sections. Theses sections were cut at 5 µm thickness on positively charged slides and incubated at 42 ºC in an oven

for one day. Then they were deparaffinized by putting them in xylene (60 min), hydrated in descending grades of alcohol and incubated in hydrogen peroxide for 5min. They were then washed in phosphate buffer saline (PBS) (5 min each). Primary antibody was applied to the slides, which were incubated for one and half hour. After that, the slides were washed two times in PBS, each slide for 5 min. Secondary antibody was applied and the sections were again incubated for 20 min, followed by washing three times in PBS for 5 min each. Diaminobenzidine tetrahydrochloride solution was applied to the sections and they were further incubated for 10 min. The sections were washed in distilled water and counterstained with Mayer's hematoxylin (2 min). Then, they were washed in tap water, dehydrated, cleared and mounted by DPX. The control group was processed following the same protocol, but without the use of the primary antibody (2) Slides were examined with an Olympus optical microscope (Olympus, Center Valley, PA, USA) equipped with an Olympus U-CMAD3 digital camera interfaced to a computer. Quantification of the caspase 3 protein expression was performed by computer-assisted Image J software (NIH, Bethesda, MD, USA).

Statistical analysis:

The data were analyzed using SPSS program version 16 (SPSS Inc., Chicago, USA). Statistical analysis was carried out using an analysis of variance (one-way ANOVA) followed by Tukeys multiple comparison test. survival analysis was done followed by Gehan-Breslow-Wilcoxon test. Correlations between data were determined with the Pearson's rank correlation coefficient. Analysis of the receiver operator characteristics (ROC) and calculation of the area under the curve (AUC) were used to evaluate the capability of serum procalcitonin . Results were expressed as mean ± SD. Values of $P \leq 0.05$ were regarded statistically significant.

Results:

Results of survival of the two different groups (figure 1):

A total of 40 rats were used in the survival experiments that were conducted independently over a period of two days. Compared to control rats (n = 20), which had a survival of 100% throughout the experimental timeline, LPS injected rats had a survival rate of 76.47% after the first day and 47.05% at the end of the second day.Gehan-Breslow-Wilcoxon test showed a highly significant decrease in the survival rate (p value is 0.0002) in LPS injected group when compared with the control group.

Diagnostic value of PCT(table 3):

Receiver operating characteristics (ROC) curve for sensitivity and specificity of serum procalcitonin. At a cutoff value of 68.75 mg/dl, PCT was shown to have 100% specificity and 100% sensitivity for detecting PCT [Area under the receiver operating characteristics curve (AUC)= 1.0 with negative and positive predictive values for PCT 100%].

Results of mouse clinical assessment score for sepsis (M-CASS) (table 2):

Rats in the LPS injected group developed highly significant (p value < 0.0001) behavioral changes including Piloerection, hunched with

severe splinting, severe obvious grimacing, lack of grooming, reduced activity, moderate to severe diarrhea and dyspnea when compared to rats of the control group which had normal behavioral changes(table 2).

Results of vital signs (figure 2):

On observing vital signs, rats intraperitoneally injected with LPS had significant physiologic derangements compared with control animals including weight loss, hypothermia, hypotension and tachycardia .They showed highly significant decrease (p value = < 0.0001) in their rectal temperature (35.07 ± 0.0801 C° versus 36.93 ± 0.277 C° respectively)(fig.2 D) MSBP (95.85 ± 3.937 mmHg versus 120.4 ± 3.789 mmHg, respectively)   , MDBP (61.70 ± 2.179 mmHg versus 71.90 ± 1.804 mmHg ,respectively) when compared to the control group(fig.2 B,C). Meanwhile, heart rate was highly significantly increase (p value = < 0.0001) in the rats of sepsis group in comparison to control group (450.5±9.411 bpm versus 333.1±10.48 bpm, respectively) (fig.2 H).

Results of biochemical blood measurements (figure 2):

Surprisingly rats in the LPS injected group, showed no signs of hyperglycemia, a common feature of human sepsis associated with stress mediated gluconeogenesis. In fact, the Tukey's Multiple Comparison Test, showed that there was hypoglycemia. Blood glucose measured was highly significantly decreased (p value = < 0.0001) in LPS injected group when compared to control group (1.705 ± 0.4383 mg/dl versus 88.05 ± 8.696 mg/dl, respectively) (fig.2 I).

Highly significant elevations (p value = < 0.0001) in serum urea and serum creatinine noticed in post-

hoc tests done between LPS injected group and control group. Serum urea was 50.70 ± 2.209 mg/dl in LPS injected group and 24.54 ± 1.463 mg/dl in the control group. While, Serum creatinine was 1.156 ± 0.258 mg/dl in LPS injected group and 0.743 ± 0.0390 mg/dl in the control group (fig.2 E, F).

Rats injected with LPS developed significant neutropenia (p value = < 0.0001), when compared to control rats (1.705±0.4383 ($\times10^3$ cells/µL) versus 3.805 ±1.419 ($\times10^3$ cells/µL) respectively (fig.2 A).

On measuring the serum Procalcitonin level, rats intraperitoneally injected with LPS had highly significant increase (p value = < 0.0001) when compared to the control group (180.8±34.17 ng/mL versus 121.4±26.23 ng/mL, respectively) (fig.2 G).

There was a negative non-significant correlation between serum Procalcitonin level and WBCs count in LPS injected rats (r = -0.0633) (fig.3 A).; while there was a positive non-significant correlation between serum Procalcitonin level and rectal temperature in LPS injected rats (r = 0.0516) (fig.3 B).

Results of imunohistochemical study:

Immunohistochemical staining was used to analyze the expression of caspase-3 proteins in the kidney tissues of the different studied groups using image J program. LPS injected group exhibited an intense significantly higher expression of caspase-3 (85.44 ± 14.18) in the cytoplasm of the tubular cells and in the glomeruli as compared with control group (**p = 0.025**)(figure 4).

Table 2 showing mouse clinical assessment score for sepsis (M-CASS) done by Kruskal-Wallis test followed by Dunn's Multiple Comparison Test .Data were expressed mean ± SD. P value ≤ 0.05 are statistically significant.

Mean ±SD	Control group (n=20)	Sepsis group (n=20)	P value
Fur aspect	1	3.65 ± 0.489	< 0.0001 ***
Activity	1	3.70 ± 0.470	< 0.0001 ***
Behavior	1.15 ±0.366	3.80 ± 0.410	< 0.0001 ***
Face	1.50 ± 0.513	4	< 0.0001 ***
Diarrhea	1.25 ± 0.444	3.75 ±0.444	< 0.0001 ***
Respiratory distress(dyspnea)	1	3.20 ± 0.410	< 0.0001 ***

Table 3 showing cut off point of sensitivity and specificity serum procalcitonin level in LPS injected group:

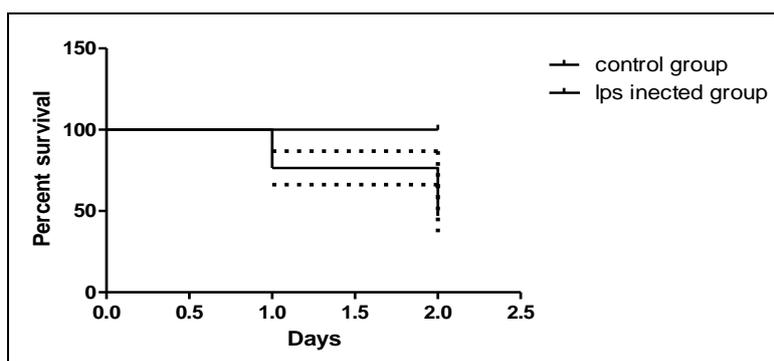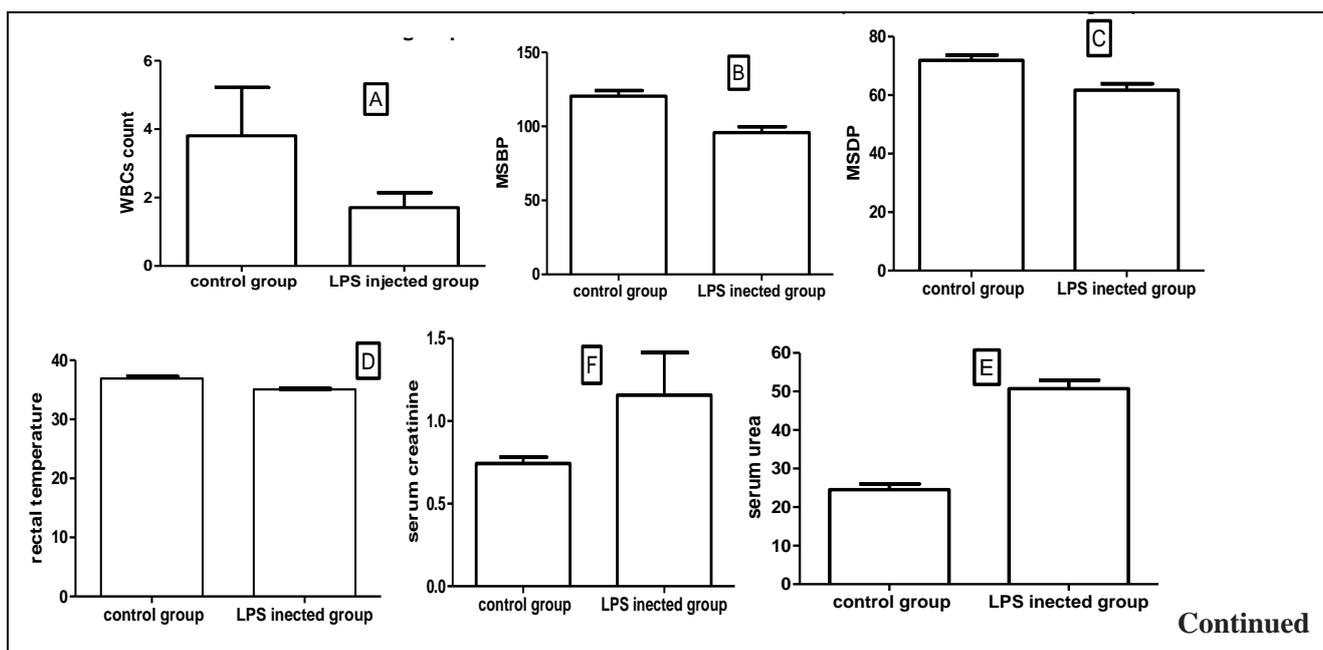| Area under the curve	95% confidence interval	Cut off point	Sensitivity%	Specificity%
1.000	1.000 to 1.000	> 68.75	100.0	100.0

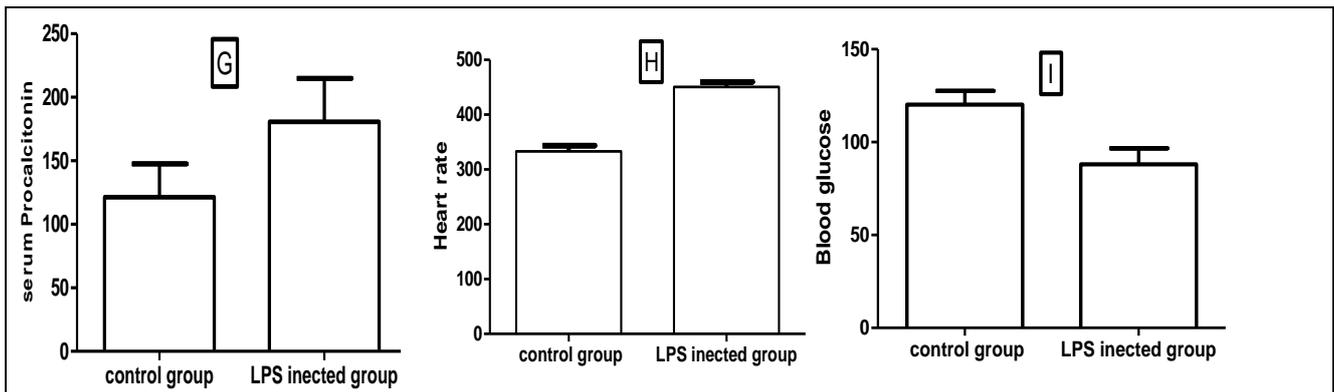Figure 1 showing survival rate of the control and LPS injected groups

Continued

Figure2 showing changes in vital signs (MSBP, MDBP, heart rate and rectal body temperature) and biochemical parameters (WBCs count, serum urea, serum creatinine, blood glucose and serum Procalcitonin) in the two different studied groups. Data were expressed mean ± SD. (WBCs=white blood cells, MSBP=mean systolic blood pressure, MDBP=mean diastolic blood pressure)
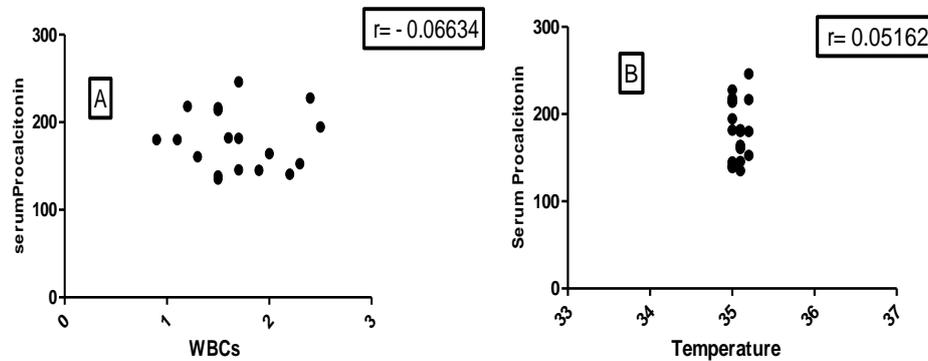

Fig (3): Correlation between serum procalcitonin level and WBCs count (A) and rectal temperature (B) in LPS injected rats.
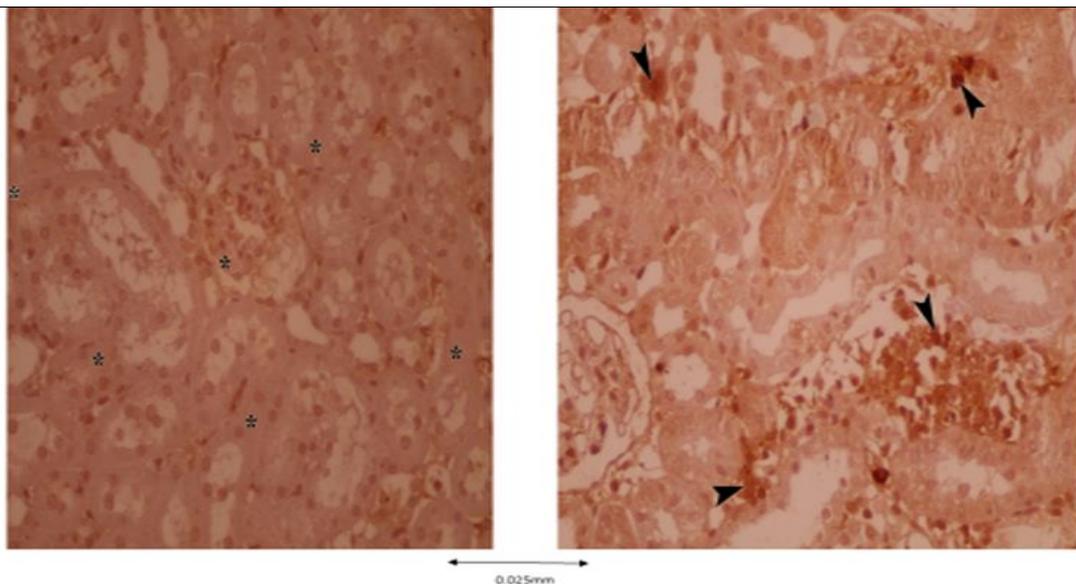

Fig 4 :Immunohistochemical staining of caspase 3-cellular expression in renal tissue in control and LPS injected groups. The control group shows a slight positive caspase 3 cellular expression (star); The LPS injected group shows an increased positive caspase 3-cellular expression (arrowhead) in glomerular & tubular cells; Magnification: × 400; scale bars: 0.25 mm.

Discussion

Sepsis is a considerable global problem and must be diagnosed and treated as soon as possible (55). it include two main stages: an introductory proinflammatory burst responsible for hypotension and organ dysfunction, followed by a compensatory anti-inflammatory reaction that leads to an immunosuppressed state frequently called immune depression or immune dysfunction; in any case, these stages can overlap each other. The latter consists of altered monocyte antigen presentation, decreased lymphocyte proliferation and responsiveness, and lymphocyte apoptosis (13, 38), which accounts for nosocomial infections and late deaths in sepsis.

In the current study, we randomized 40 adult male Sprague–Dawley rats and divided them equally into 2 groups control and sepsis by LPS. In this proof-of-concept study, our primary endpoint was survival to 48 h after LPS injection. During the course of the study; rats behavior (rat fur, activity, face grimacing, occurrence of diarrhea and respiratory distress was assessed, vital signs (temperature, mean systemic systolic &diastolic blood pressure and heart rate)were measured and laboratory blood tests (WBCs count ,serum urea &creatinine and serum procalcitonin) were determined. The study aimed to analyze the serum procalcitonin levels as a specific accurate biomarker for detection of bacterial infection in LPS rat model of sepsis.

On the 48 hours observation of the LPS rats ;the rats developed highly significant (p value < 0.0001) behavioral changes including piloerection, hunched with severe splinting, severe obvious grimacing, lack of grooming, reduced activity, moderate to severe diarrhea and dyspnea when compared to rats of the control groups which had normal behavioral changes. This goes with pervious study done by (25); they stated that sepsis significantly decreased the total distance, speed, rearing/leaning and number of grooming events rats can perform normally. Tuon *et al.* (53) also demonstrated in sepsis-surviving rats, a significant increase in the immobility time as compared to the sham rats. The expert working group (EWG) consisting of veterinarians, animal technologists, and scientists gathered in 2014 stated that weight loss, lack of voluntary movement, diarrhea (51)and facial expression (18)are the main indicators of suffering in sepsis rat model (11)and the main determining factor to end the study .They referred to it as a humane end point which is bunch of criteria that empowers a study to be finished earlier or to reduce pain or trouble so that the enduring of the animals can be diminished or stopped humanly (52).

This may be due to imbalance of oxidants and antioxidants, assist advancing cell death as well as tissue harm in vulnerable regions of the brain, mainly the hippocampus (43). Enactment of systemic inflammation by LPS is to a great extent interceded by means of interaction of the bacterial item with Toll-like receptor (TLR) 4, which is located on the surface of both "professional" immune cells, such as monocytes and macrophages, as well as numerous other cell types, as alveolar epithelial cells and myocardial cells (28).

The highly significant hypothermia notice d in LPS injected rats matches the results of previous studies done by (11). They suggested that

hypothermia related with septic condition is a versatile and defensive reaction to sepsis. Septic animals generally shut down and gotten to be exceptionally cold, nearly resting (state of torpor) (58). Without a doubt, it has been recommended that hypometabolism and resulting hypothermia may anticipate hypoxia in a rodent model of endotoxic shock (10). Interests, hypothermia causes upregulation of anti-inflammatory IL-22 (8) and IL-10 (42) in cases of systemic inflammatory reaction syndrome/endotoxemia (14).

Significant hemodynamic changes noticed in LPS injected group when compared with control animals including hypotension and tachycardia was noticed in the current study and can be easily explained by Mary's law of the heart which stated that heart rate is inversely proportional to arterial blood pressure which was formulated in 1861 .Also, these hemodynamic changes go with the pervious results mentioned by (35).

They expressed that sepsis significantly increase heart rate and significantly diminish stroke volume and cardiac output due to diminished cardiomyocytes oxidative capacity and myocardial damage which at long last influence cruel blood vessel blood pressure causing hypotension. Meanwhile, sepsis as a rule associated chloride toxicity due to sepsis initiated renal failure which inhibit the renin angiotensin system (34). They noticed that blood vessel hypotension is common in patients with septic shock and extreme sepsis, due to hypovolemia and blood vessel vasodilation, and it is the most cause of organ hypoperfusion and eventually to organ failure (34).Interestingly, rats in the LPS injected group, showed signs of hypoglycemia with significant decrease in blood glucose level. This in

accordance of pervious study which showed that all rats administered LPS, developed hypoglycemia or remained in the lower range of normoglycemia (62). They stated that higher levels of IL-1 and TNFα associated with septic condition may be a cause of hypoglycemia seen in sepsis rat model. This may be related to decreased gluconeogenesis, and connected to diminish mitochondrial phosphoenolpyruvate carboxykinase (7).

In spite of the fact that leukocytosis is the usual reaction to bacterial contamination (50), LPS injected rats within the display study showed neutropenia. It may appear in septic animals and people, auxiliary to upgraded leukosequestration at the site of infection (location of LPS injection in our demonstrate) or inside the distinctive organs, as a forerunner to sepsis-associated intense organ failure, and is indicative of more regrettable outcome (62).

Highly significant elevations (p value = < 0.0001) in serum urea and serum creatinine noticed in post-hoc tests done between LPS injected group and control group. This goes with pervious study done by Nishkantha *et al*, 2018. They noticed rise in serum creatinine in septic rodent model. Acute kidney injury is diagnosed by a sudden diminish in glomerular filtration rate (GFR), the primary measure of kidney function, which is as of now recognized clinically as a rise in serum creatinine. Several different pathophysiological mechanisms have been proposed for sepsis-induced AKI: vasodilation-induced glomerular hypoperfusion, dysregulated circulation within the peritubular capillary network, inflammatory reactions by systemic cytokine storm or local cytokine production (44),

and tubular dysfunction induced by oxidative stress (61). Pervious theory tried by Kent *et al* (17), evaluated that AKI may be a effective adjustment that secures the body from losing sodium by using tubuloglomerular feedback (TGF) to diminish GFR (17).

On measuring the serum Procalcitonin level, rats intraperitoneally injected with LPS had highly significant increase (p value = < 0.0001) when compared to the control group .PCT is a host-response marker that is up-regulated by microbial toxins and certain proinflammatory mediators (e.g., interleukin-1b, tumor necrosis factor-α, interleukin-6) and is down-regulated during recovery (45). The expression of PCT is weakened by the cytokines ordinarily discharged in reaction to a viral contamination (e.g., interferon-γ); in this way, an raised PCT is ordinarily characteristic of a bacterial infection (21). In expansion to its diagnostic esteem, the kinetics of PCT have moreover been appeared to anticipate mortality and treatment failure in sepsis (16, 33). PCT has produced much interest as a sepsis biomarker that's associated with expanded hazard and seriousness of bacterial contamination. PCT has moreover been found to relate with hazard of culture-proven bacteremia (19). In spite of the fact that the essential function of PCT in host defense is not entirely caught on, this peptide impacts the safe framework of immunity in different ways counting a diminish in phagocytic and candidacidal action of neutrophils additionally leads to an increment within the concentration of intracellular calcium particles (9,3)which encourage the host reaction. Imperatively, past investigate illustrates that PCT can be used to inform anti- microbial choices, primarily by

lessening anti-microbial initiation in low- risk patients (i.e., bronchitis, upper respiratory disease) and by early stoppage of anti-microbials in patients with pneumonia and sepsis (6, 45 ,46).Results of the sensitivity and specificity of serum procalcitonin showed that at a cutoff value of 68.75 mg/dl, PCT was shown to have 100% specificity and 100% sensitivity for detecting PCT which indicate that the serum PCT is highly specific and highly sensitive and could be the most reliable method for diagnosis of the septic conditions.

The increase expression of caspase-3 in the cytoplasm of the tubular cells and in the glomeruli in LPS injected group in the current study goes with Messaris *et al.* (26) who observed that apoptotic renal tubular cell death was increased significantly 6 hours after sepsis induction and declined afterwards. Apoptosis is a rapid process, taking approximately 4–6 hours from initiation to the structural disassembly of apoptotic cell (49). Aunapuu *et al.* (1) demonstrated that histologically changes in kidneys start in 2 hours after *Escherichia coli* injection. Caspase cleaved cytokeratin 18 (CK18) is an early marker of apoptosis. It can be recognized in epithelial cells using a monoclonal antibody (M30) directed against this neoepitope. CK18 is cleaved by several caspases (caspase-3, -6, -7, and -9) during apoptosis, and is dependent of single caspase activation (56). Since M30 neoepitope occurs in the early stage of the apoptotic cascade, it can be applied in the determination of induction or suppression of the programmed cell death process (40).

Conclusion :

This study prove that serum procalcitonin is a noval accurate and specific biomarker that shows efficacy in the diagnosis of sepsis and its associated renal dysfunction, which explained by apoptosis and the increase in renal tissue caspase 3 expression.

Refernces:

1. **Aunapuu, K, Tapfer, et al** ,2004. Morphological and ultrastructural changes in the rat kidney after experimental sepsis, *Tsitologiia*, vol. 46, no. 8, pp. 690–694.

2. **Bancroft, J. and Gamble M**., 2002: Theory and practice of histological techniques .*5th ed. Philadelphia: Churchill Livingstone*.

3. **Becker KL, Snider R, Nylen ES** ,2010 Procalcitonin in sepsis and systemic inflammation: A harmful biomarker and a therapeutic target. *Br J Pharmacol*; 159:253–264

4. **Bienvenu J, Monneret G.** ,2001 Procalcitonin as an acute phase marker. *Ann Clin Biochem*; 38:483–93.

5. **Bone RC, Sprung CL, Sibbald WJ.** 1992. Definitions for sepsis and organ failure. *Crit Care Med* 20:724–726.

6. **Bouadma L, Luyt CE, Tubach F, et al**, 2010 PRORATA trial group: Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): A multicentre randomised controlled trial. *Lancet*; 375:463–474

7. **Caton PW, Navuni NK, Murch O, et al.** ,2009 Endotoxin induced hyperlactatemia and hypoglycemia is linked to decreased mitochondrial phosphoenolpyruvate carboxykinase. *Life Sci*;84(21–22):738–744.

8. **Chichelnitskiy E, Himmelseher B, Bachmann M, et al** ,2017 Hypothermia Promotes Interleukin-22 Expression and Fine-Tunes Its Biological Activity. *Front. Immunol*. 8:742.

9. **Christ M, Müller B**,2008 Calcitonin peptides–the mediators in sepsis or just another fairy tale? *Crit Care Med*; 36:1684–1687

10. **Corrigan J, Fonseca M, Flatow E, et al** ,2014 Hypometabolism and hypothermia in the rat model of endotoxic shock: independence of circulatory hypoxia. *J Physiol* 592(17):3901Y3916.

11. **Elliot L, Rachel A, Nicole C, et al .**,2015 refinement of animal models of sepsis and septic shock. *SHOCK*, Vol. 43, No. 4, pp. 304Y316.

12. **Hatzistilianou M**,2010. Diagnostic and prognostic role of procalcitonin in infections.*Scientific World Journal*; 10:1941-6.

13. **Hotchkiss, R, and Karl,** 2003. The pathophysiology and treatment of sepsis. *N. Engl. J. Med*.348:138–150.

14. **Huet O, Kinirons B, Dupic L, et al**,2007 Induced mild hypothermia reduces mortality during acute inflammation in rats. *Acta Anaesthesiol Scand* 51:1211–6. doi:10.1111/j.1399-6576.2007.01419.x

15. **Husova L, Husa P, Senkyrik M,et al** ,2004. Procalcitonin as an indicator of infection in patients with liver cirrhosis. *Vnitr Lek* Feb;50(2):153-6.

16. **Jensen JU, Hein L, Lundgren B, et al**,2011 Procalcitonin And Survival Study (PASS) Group: Procalcitonin-guided interventions

against infections to increase early appropriate antibiotics and improve survival in the intensive care unit: A randomized trial. *Crit Care Med*; 39:2048–2058

17. <u>Kent D</u>, <u>Asada L, Peter S</u> , 2009 Animal models of sepsis and sepsis-induced kidney injury .*Clin Invest.;*119(10):2868-2878.

18. **Langford D, Bailey A, Chanda M, et al.** ,2010 Coding of facial expressions of pain in the laboratory mouse. *Nat Methods* 7:447Y449.

19. **Laukemann S, Kasper N, Kulkarni P, et al** ,2015 Can we reduce negative blood cultures with clinical scores and blood markers? results from an observational cohort study. *Medicine (Baltimore);* 94:e2264

20. **Lilley E, Armstrong R, Clark N, et al.**,2015 Refinement of animal models of sepsis and septic shock. *Shock* 43(4):304–316.

21. **Linscheid P, Seboek D, Schaer DJ, et al** ,2004 Expression and secretion of procalcitonin and calcitonin gene-related peptide by adherent monocytes and by macrophage-activated adipocytes. *Crit Care Med* ; 32:1715–1721

22. **Martin GS, Mannino DM, Eaton S** ,2003. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*; 348:1546–54.

23. **Martin, G.S.** 2012. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. *Expert Review of Anti-Infective Therapy* 10(6): 701–6.

24. **Martin, Hagimato, Nakamura, et al** ,2005 Apoptosis and epithelial injury in the lungs, *Proceedings of the American Thoracic Society*, vol. 2, pp. 214–220.

25. **Mengrao S, Chunxiu Z, Xuhua G, et al**,2018 .Berberine Prevents Cognitive Disorders Induced by Sepsis by Regulating the Inflammatory Cytokines, Oxidative Stress and Neuronal Apoptosis in Rat Brain. *Neuropsychiatry* Volume 8, Issue 1

26. **Messaris, Memos, Chatzigianni et al.,** 2008 Apoptotic death of renal tubular cells in experimental sepsis, *Surgical Infections*, vol. 9, no. 3, pp. 377–388.

27. **Michalik DE, Duncan BW, Mee RBB**. ,2006 Quantitative analysis of procalcitonin after pediatriccardiothoracic surgery. *Cardiol Young*;16:48–53.

28. **Mitchell P** ,2014 Animal models of sepsis. *Virulence* 5:1, 143–153

29. **Morgenthaler NG, Struck J, Chancerelle Y.** .2003 Production of procalcitonin (PCT) in non thyroidal tissue after LPS injection.Horm *Metab Res*;35: 290–295.

30. **Nishkantha, Arulkumaran, Pollen** .2018 Renal Tubular Cell Mitochondrial Dysfunction Occurs Despite Preserved Renal Oxygen Delivery in Experimental Septic Acute Kidney Injury *Critical Care Medicine:* Volume 46 - Issue 4 - p e318–e325

31. **Ofer Y, Adam L, Kelly K** ,2015 Effects of exendin-4, a glucagon like peptide-1 receptor agonist, on neutrophil count and inflammatory cytokines in a rat model of endotoxemia . *Journal of Inflammation Research*:8 129–135

32. **Oshita H, Sakurai J, Kamitsuna M.** ,2010 Semi-quantitative procalcitonin test for the diagnosis of bacterial infection: clinical use and experience in Japan. *J Microbial Immunol Infect*;43(3):222-7.

33. **Pieralli F, Vannucchi V, Mancini A, et al**, 2015 Procalcitonin kinetics in the first 72 hours predicts 30-day mortality in severely ill septic patients admitted to an intermediate care unit. *J Clin Med Res* ; 7:706–713

34. **Pierre O, François B, Valérie S,et al** ,2017 Assessment of renal hemodynamic toxicity of fluid challenge with 0.9% NaCl compared to balanced crystalloid (PlasmaLyte®) in a rat model with severe sepsis .*Annals of Intensive Care* 7:66

35. **Pinto, Bernardo B., Dyson, et al** 2017 Improved Survival in a Long-Term Rat Model of Sepsis Is Associated With Reduced Mitochondrial Calcium Uptake Despite Increased Energetic Demand *Critical Care Medicine*. 45(8):e840-e848.

36. **Rahimkhani M, Ghofrani H.** 2008 Helicobacterpylori and peptic ulcer in cirrhotic patients. *Pak J Med Scien*;24(6):84952.

37. **Rahimkhani M, Khavari H, Jamali S** 2011. Serologic evaluation of hepatitis B and D in patients with cirrhosis. *African J Microb Research*; 5(5):568-571.

38. **Riedemann, N, Guo, and Ward** ,2003. Novel strategies for the treatment of sepsis. *Nat.Med.* 9:517–524.

39. **Ronald C,John B, Holcomb** ,2018 plasma resuscitation improved survival in a cecal ligation and puncture rat model of sepsis. *shock*, vol. 49, no. 1, PP. 53–61

40. **Roth, Krenn, Brunner et al.,** 2004 Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope M30 in critically ill patients," *Shock*, vol. 22, no. 3, pp. 218–220.

41. **Russwurm S, Stonans I, Stonane E, et al.** ,2001 Procalcitonin and CGRP-1 mRNA expression in Various human tissues. *Shock*;16:109–12.

42. **Sabat R, Grütz G, Warszawska K, et al**, 2010. Biology of interleukin-10. Cytokine Growth Factor *Rev* 21:331–44. doi:10.1016/j.cytogfr.2010.09.002

43. **Salvemini D, Cuzzocrea S.** ,2002 Oxidative stress in septic shock and disseminated intravascular coagulation. *Free. Radic. Biol. Med* 33(9), 1173-1185.

44. **Schrier, R, and Wang, W** ,2004 Acute renal failure and sepsis. *N. Engl. J. Med.* 351:159–169.

45. **Schuetz P, Albrich W, Mueller B** 2011 Procalcitonin for diagnosis of infection and guide to antibiotic decisions: Past, present and future. *BMC Med*; 9:107

46. **Schuetz P, Briel M, Christ-Crain M, et al** ,2012 Procalcitonin to guide initiation and duration of antibiotic treatment in acute respiratory infections: An individual patient data meta-analysis. *Clin Infect Dis*; 55:651–662

47. **Schuetz P, Chiappa V, Briel M, et al** ,2011 Procalcitonin algorithms for antibiotic therapy decisions: A systematic review of randomized controlled trials and recommendations for clinical algorithms. *Arch Intern Med*; 171:1322–1331

48. **Seymour CW, Liu VX, Iwashyna TJ, et al** ,2016 Assessment of clinical criteria for sepsis: for the third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315(8):762–774,

49. **Shaker and Sadik** ,2013 .Pathogenesis of preeclampsia: Implications of apoptotic markers and oxidative stress .*Human & Experimental Toxicology* ,Vol 32, Issue 11, pp. 1170 - 1178

50. **Shi Y, Jinyoung K, Hong B, et al** ,2018 Acute and Critical Care ,33(1):34-41

51. **Sotocinal S, Sorge R, Zaloum A, et al.**, 2011 The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain* 7(1):55Y65.

52. **Stokes W**, 2000 Reducing unrelieved pain and distress in laboratory animals using humane endpoints. *ILAR J* 41:59Y61.

53. **Tuon L, Comim CM, Antunes MM, et al** ,2007. Imipramine reverses the depressive symptoms in sepsis-surviving rats. *Inten. Care Med*. 33:2165-2167.

54. <u>**Uğur K**</u> **, Çimen G ,Olguner, et al** ,2013 The Effects of Dexmedetomidine on Secondary Acute Lung and Kidney Injuries in the Rat Model of Intra-Abdominal Sepsis *The Scientific World Journal* , Article ID 292687, 11 pages

55. **van der Poll T, van de Veerdonk FL, Scicluna BP, et al.** ,2017 The immunopathology of sepsis and potential therapeutic targets. *Nat. Rev. Immunol* 17(7), 407-420

56. **van Grieken, Meijer, Zur Hausen, et al** ,2003 Increased apoptosis in gastric mucosa adjacent to intestinal metaplasia, *Journal of Clinical Pathology*, vol. 56, no. 5, pp. 358–362.

57. **Wang, L., Chen, Wang**,2014. Protective effect of quercetin on lipopolysaccharide-induced acute lung injury inmice by inhibiting inflammatory cell influx. *Experimental Biology and Medicine* 239(12): 1653–1662.

58. **Wendon J**,2010: Critical care Bnormality[: individualized verses protocolized care. *Crit Care Med* 38(10):S590YS599.

59. **Wichterman KA, Baue AE, Chaudry IH.** ,1980 . Sepsis and septic shock: a review of laboratory models and a proposal. *J Surg Res* 29:189–201.

60. **World Health Organization (WHO).** 1999 WHO report on infectious disease: removingobstacles to healthy development.*Geneva:WHO,*.www.who.int/infectious-disease-report/index

61. **Wu L., Gokden, N., and Mayeux** , 2007. Evidence for the role of reactive nitrogen species in polymicrobial sepsis-induced renal peritubular capillary dysfunction and tubular injury. *J. Am. Soc. Nephrol*. 18:1807–1815.

62. **Yanay O, Bailey A, Kernan K, et al** ,2015 Effects of exendin-4, a glucagon like peptide-1 receptor agonist, on neutrophil count and inflammatory cytokines in a rat model of endotoxemia*. Journal of Inflammation Research*:8 129–13