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IMPACT OF BACTERIAL LIPOPOLYSACCHARIDE ON CYTOKINES RESPONSES AT PREGNANCY

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Abstract

Cytokines mediate and regulate inflammatory and immune responses. The purpose of this study was to investigate the effect of lipopolysaccharide (LPS) and inflammatory cytokines on pregnancy. Mice were intraperitoneal injected with sub LD_{50} of LPS at different gestational (early, mid, late) stages, firstly the LD_{50} of LPS were determined. Animals were treated with single sub LD_{50} dose of LPS via intraperitoneal challenge. Levels of cytokines such as IL-1and IL-2 were estimated in animal sera at different times after LPS challenge by ELISA. There was significant difference between the sera levels of IL-1, IL2 for pregnant mice treated with LPS and controls.
***Keywords*:** Lipopolysaccharide, LD_{50} of LPS, Murine model, Cytokine response, IL-1, IL-2

Introduction

Lipopolysaccharide (LPS) is the outer component of the outer membrane (OM) in Gram-negative bacteria and frequently plays a key role in pathogenesis (Whitfield and Trent, 2014), It is the dominant glycolipid in the outer leaflet of the outer membrane forming a layer that is stabilized by divalent cations and provides an effective permeability barrier against deleterious molecules such as antibiotic (Nikaido, 2003). LPS molecule has a tripartite structure comprising lipid A, core oligosaccharide and O antigen polysaccharide or O antigen (Whitfield and Trent, 2014). LPS releases from the bacteria during cell division, cell death, or in particular, as a result of antibiotic treatment against bacterial infection (Raetz and Whitfield, 2002). Upon release LPS is recognized by mononuclear phagocytes (monocytes and macrophages), which are part of the innate immunity of the host, and activates them. This results in an increase in their phagocytic activity and significantly enhances the secretion of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6), IL8, IL4, IL1, IL2 and others (Dobrovolskaia and Vogel, 2002). Tissues recognize bacterial toxins by specific pattern recognition receptors, including Toll_like receptors (TLRs) (Hirsch and Wang, 2005). TLR4 is the major LPS signaling receptor in mammals (Hoshino *et al.*, 1999). LPS recognition and responsiveness is strongly enhanced when LPS binds with cluster of differentiation 14 (CD14). Since CD14 has no ability to transduce a signal, it facilitates association of LPS with TLR4 (Nagai *et al.*, 2002). TLR4 mediates LPS actions via cytosolic adaptor protein myeloid differentiation factor 88 (MyD88) or Toll-interleukin-1 receptor (TIR) domain-containing adaptor-inducing interferon-β (TRIF) (Yamamoto *et al.*, 2003). Typical inflammatory responses to LPS via TLR4-MyD88 pathway includes production of proinflammatory molecules such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL6, and type II interferon (INF), IFN-γ. On the other hand, TLR4-TRIF signaling is primarily responsible for inducing production of type 1 interferons, IFNα and INFβ, and IFN-stimulated genes (Takeda and Akira, 2005). Endometrial and decidual cells of women and mice express TLR4 and respond to LPS (Sheldon and Roberts, 2010) and TLR4-MyD88 pathway promotes sharp inflammation in the uterus in response to LPS. Despite these progresses in our understanding of the infection-induced inflammation response, maternal infection remains a major contributor of pregnancy complications (Cronin *et al.*, 2012) . Interleukin-10 (IL-10) is an anti-inflammatory cytokine which minimizes pregnancy-related inflammation through regulation of TNF-α and other cytokines and chemokines (Moore *et al.*, 2001). Cytokines and metabolites, when produced in moderate amounts, initiate beneficial anti-infectious inflammatory reactions of the host, e.g. moderate fever, activation of defense cells and microbicidal mechanisms and initiation of acute phase reactions. However, high amounts of these products have harmful effects on the host, inducing various fatal pathophysiological effects such as high fever, hypotension, disseminated intravascular coagulation and damage of cells and organs, which results in the manifestation of septicaemia, multi organ failure and lethal septic shock (Takeda and Akira, 2005). The exposure of pregnant mice to LPS leads to embryonic loss. The endotoxin-induced pregnancy loss may be mediated by the release of various proinflammatory cytokines/growth factors. These factors may alter the normal physiology of the reproductive organs of the mother, and may change the delicate estrogen/ progesterone ratio required for the maintenance of successful pregnancy (Baines *et al.*, 1996). The present study is carried out to determine the inflammatory that mediated by cytokines IL1and IL2 post intra peritoneal injection of Lipopolysaccharide in pregnant mice.

Materials and Methods

LPS: Lipopolysaccharide of E .coli serotype O111:B4 (Sigma) was used for induction of animal sepsis

Lab. animals: Sexual female mice (20-25gm) weight and (8-12weeks) age the mice were placed in well ventilated wire-plastic cages and left for 21days to acclimatize under controlled conditions about 12 hour light and 12 hour dark temperature was set at 18-20°C. Animals were given water and proper food

Experiment design: Mice were grouped as follow: Groups C1, C2 and C3: Mice were given normal saline as controls. Groups T1, T2 and T3: Mice were intra peritoneal injection of Lipopolysaccharide mice. mice divided randomly to six groups named as C1 control group at first weeks of

pregnancy stage, C2 control group at second weeks of pregnancy stage and C3 control group at third weeks of pregnancy stage these group given normal saline only.T1Group at first weeks of pregnancy stage, T2 group at second weeks of pregnancy stage and T3 at third weeks of pregnancy stage these group Given intraperitonaly LPS (125µg/kg). Animals were scarified at times (0, 2 ,6, 12 and 24) after LPS injection. Blood was collected by heart puncture then sera obtained for evaluation of the IL1, IL-2 concentration.

Measurement of cytokine serum levels:

IL-1 and IL-2 levels were evaluated in animal sera by Enzyme Linked Immunosorbent Assay using mouse specific commercial Kit Elabscience and according to manufactural instructions

Statistical analysis:

The experiment was designed as ANOVA test was used for analysis of data that presented as mean ± SD.

Results and Discussion

Cytokine level in the sera of pregnant mice

The mean of IL-1 levels in the first stage of pregnant mice treated with LPS were higher (71.77) Pg /ml than the controls (23.96) Pg /ml which reached peak after 2hr. Statistical analysis revealed a significant difference between the sera levels of IL-1 for pregnant mice treated with LPS and controls ($p<0.05$) Fig. 1.

Fig. 1 : Serum IL -1β levels of mice group T1(n= 8 mice /group), treated as: (single dose subLD$_{50}$ of LPS), and mice group C1 (Normal saline as control). Serum levels of IL-1β was estimated at times, 2, 6, 12, and 24 hr after LPS injection in first stage of pregnancy

The mean of IL-1 levels in the second stage of pregnancy mice treated with LPS were higher (138.6) than the control ones (24.36). and reached peak after 6hr Statistical analysis revealed a significant difference between the sera levels of IL-1 for pregnant mice treated with LPS and controls ($p<0.05$). Fig. 2

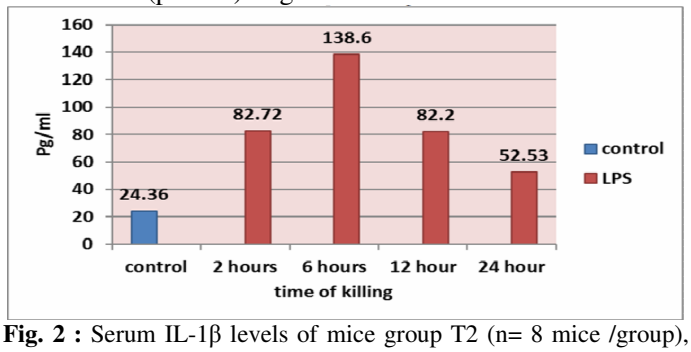

Fig. 2 : Serum IL-1β levels of mice group T2 (n= 8 mice /group), treated as: (single dose subLD$_{50}$ of LPS),and mice group C2 (Normal saline as control). Serum levels of IL-1β was estimated at times, 2, 6, 12, and 24 hr after LPS injection in second stage of pregnancy.

The mean of IL-1 levels in the third stage of pregnant mice treated with LPS was higher (95.33) than the control ones (25.15). and reached peak after 2hr. Statistical analysis revealed a significant difference between the sera levels of IL-1 for pregnant mice treated with LPS and controls ($p<0.05$). **Fig. 3**

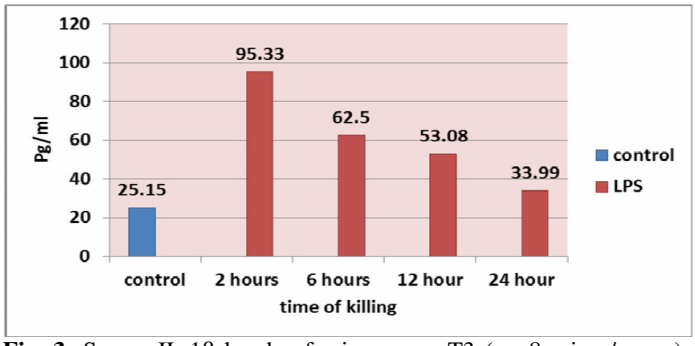

Fig. 3: Serum IL-1β levels of mice group T3 (n= 8 mice /group), treated as: (single dose subLD$_{50}$ of LPS), and mice group C3 (Normal saline as control). Serum levels of IL-1β was estimated at times, 2, 6, 12, and 24 hr after LPS injection in third stage of pregnancy.

The mean of IL-2 levels in the first stage of pregnant mice treated with LPS were higher (60.16) than the control ones (25.36). and reach to peak in 2hr. Statistical analysis revealed a significant difference between the sera levels of IL-2 for pregnant mice treated with LPS and controls ($p<0.05$). Fig.4

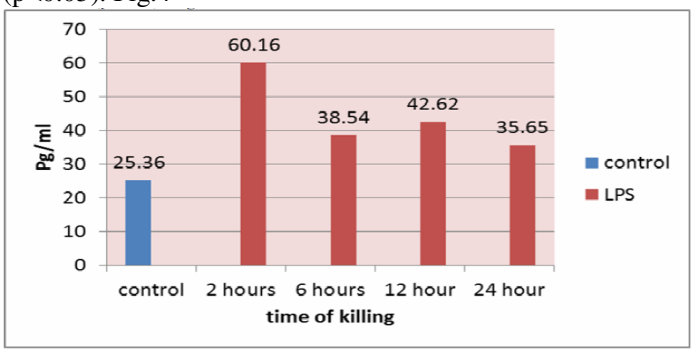

Fig. 4 : Serum IL -2 levels of mice group T1(n= 8 mice /group), treated as: (single dose subLD$_{50}$ of LPS),and mice group C1 (Normal saline as control). Serum levels of IL-2 was estimated at times, 2, 6,12 and 24 hr after LPS injection in first stage of pregnancy

The mean of IL-2 levels in the second stage of pregnant mice treated with LPS was higher (53.47) than the control ones (24.4). and reached peak after 12 hr. Statistical analysis revealed a significant difference between the sera levels of IL-2 for pregnant mice treated with LPS and controls ($p<0.05$). Fig. 5

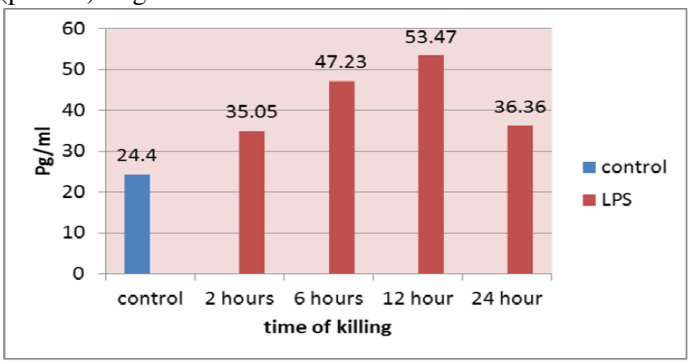

Fig. 5 : Serum IL -2 levels of mice group T2(n= 8 mice /group), treated as: (single dose subLD$_{50}$ of LPS), and mice group C2 (Normal saline as control). Serum levels of IL-2 was estimated at times,2, 6,12 and 24 hr after LPS injection in second stage of pregnancy

The mean of IL-2 levels in the third stage of pregnant mice treated with LPS was higher (48.2) than the control ones (25.17) and reach to peak in 6hr Statistical analysis revealed a significant difference between the sera levels of IL-2 for pregnant mice treated with LPS and controls ($p<0.05$). Fig. 6

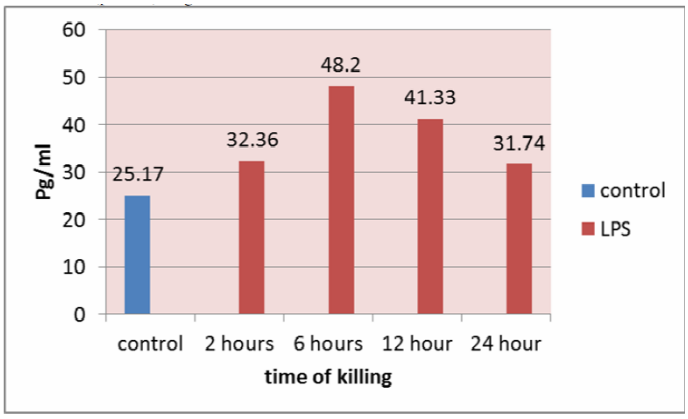

Fig. 6 : Serum IL-2 levels of mice group T3 (n= 8 mice /group), treated as: (single dose sub LD_{50} of LPS), and mice group C3 (Normal saline as control). Serum levels of IL-2 was estimated at times, 2, 6, 12 and 24 hr after LPS injection in third stage of pregnancy

The interleukin-1 system (IL-1α, IL-1β, and IL-1 receptor (IL-1R)) have previously been shown to play an important role in pregnancy of murine (Kauma, 2000a). LPS induced elevation of proinflammatory cytokine (IL-1*β*) (Ribeiro *et al.*, 2015). The biological effects of LPS are mediated by the proinflammatory cytokine (IL1) which implicated in the delicate immune system balances that exist within the feto–maternal interface (Kauma, 2000b). The intraperitoneal injection of IL-1β on day 1 of pregnancy caused 100% failure in blastocyst implantation these results indicate that elevated levels of IL-1B in response to LPS could be responsible for implantation failure and infertility in the mouse (Deb *et al.*, 2004c). The controlled expression of (IL-1β) in embryonic and uterine tissue may be essential for the successful implantation of embryos in the mouse, and any disturbance in pattern of expression of this cytokine in response to LPS may lead to failure of implantation due to increase the production of PGE2 and IL-6 from murine decidua (Dudley *et al.*, 1993).

LPS significantly increased the output of proinflammatory cytokines (IL-1B, IL-2, IL-6, IL-12, IL-15) this then leads to an over exaggerated inflammatory response potentially resulting in preterm labor (Li *et al.*, 2014). The result were in accordance with (Zhong *et al.*, 2002) which observed LPS induce NK cell infiltration and higher IL-2contents in the uterus of pregnancy mice and play important roles in fetal resorption. The LPS-induced embryo resorption has been correlated with the altered level of several other cytokines IL6, IL8, IL 2, Interferon gamma (IFNγ) (Romero *et al.*, 1990).

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