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IMMUNOHISTOCHEMICAL STUDIES ON METRITIS IN SHE-CAMELS

By

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ABSTRACT

The reproductive efficiency of dromedary camels is very low and it is a major problem in camelids. So, the aim of this study was to evaluate the uterine infection of slaughtered she-camels through identification of bacterial agents colonizing the uterine environment, with observing the uterine histopathological picture, immunohistochemical and immunological view characterization of endometritis in order to find out which factors possibly influence the progress of endometritis. A total of 100 uteri from slaughtered adult she -camels were collected for this study from Kerdassa, Nahia and El-Mounieb abattoirs. *S. aureus*, *E. coli*, *S. pyogen* and *P. multocida* were isolated in 38 samples as single or mixed infections with percentage (52.5%) and (47.5%) respectively. *S. aureus* was the most isolated bacteria as single infection with percentage of 26.2% followed by *E. coli* 15.8 % then *S. pyogen* (10.5%) while in mixed infection *S. aureus* + *E.coli* were the most prevalent mixed infection as they represented 15.8% followed by *S. aureus* + *S. pyogen* with a percentage of 10.5%. According to the histopathological examination, the affections were classified into chronic endometritis was the most common recorded endometritis type (68.4 %) followed by acute endometritis (23.7 %) then sub-acute endometritis (7.9 %). The uterus showed degenerative changes in the uterine glands with marked inflammatory cell infiltrations and edema besides congestion and vasculitis. In addition to, connective tissue proliferation and hyperplasia of endometrial mucosa were seen in chronic cases. Moreover, demonstration of immune cells in chronic endometrial speciments by immunohistochemistry revealed the distribution and type of immune cell as increasing in CD_3, CD_{20} and CD_{68} with the severity of inflammation associated with the presence of CD_{138}. By measuring the levels of some selected immunological parameters in the blood serum samples as biomarkers for the immune status of both she-camels with sub-acute, acute endometritis NO, Hp and SAA concentrations were significantly higher in serum samples of she-camels with acute endometritis than that of

sub-acute endometritis, and in the sub-acute cases when compared with the samples of apparently normal she-camels. The dendrogram analysis illustrated that there were weak similarities in the protein fingerprints between samples from she-camels infected with sub-acute endometritis and that of apparently normal she-camels ranging from (0.25 - 0.33).

Key words:

She-camels-Bacteria- Histopathology-Immunohistochemistry-Acute Phase Protein-Lysozyme.

INTRODUCTION

Camels (*Camelus dromedarius*) are hardy animals and less susceptible to diseases that affect cattle, but little is known about the diseases they suffer from **(Jenberie *et al*., 2012).** The reproductive efficiency of dromedary camels is very low and it is a major problem in camelids. The short breeding season is an important factor for the low reproductive performance of dromedary camels which is due to shortage of food. Pathological abnormalities of female reproductive tract have also been reported as the main causes of infertility **(Melaku *et al*., 2015).** Like so many domestic animal species, uterine infections are the most common of these disorders, but unlike other species, little is known about their pathogenesis and evolution in camelids **(Tibary, 2001).** Generally, infection of the genitalia during the peripartum period leads to metritis and endometritis **(Mshelia *et al.*, 2013)** which are probably associated with substantial economic losses in livestock production **(Shokri *et al.*, 2010)**, majorly through prolonged calving intervals, repeat breeding and abortion, reduced milk production, and culling **(Tibary *et al.*, 2006),** in addition to, high rate early embryonic loss (**Moustafa *et al*., 2004).** Different uterine affections have been described in the camel with some reports of bacterial causative agents such as *Arcanobacterium pyogenes*, *S. pyogenes*, *S. aureus*, *Corynebacterium* and *E. coli* as well as *Proteus* was frequently isolated from she camels with uterine infections **(Ali *et al.,* 2010 and Nabih and Osman 2012).** Until now, more than thirty-five bacteria species are known infecting the genital tract after calving **(Dolatkhah *et al.,* 2013).** Moreover, **Al-Humam (2016)** recorded that *E. coli*, *A. pyogenes*, *Mannheimia morgana* and *Cand. albicans* were mainly associated with purulent endometritis.Since reproductive system disorders including uterine infections are so important, therefore, much more scientific reports have been published especially in fields such as metritis, endometritis and pyometra which are the most common uterine disorders **(Youngquist and Threlfall,2007).** Following parturition, natural mating, artificial insemination and infusion of irritant materials into the uterus, endometritis may occur which can be diagnosed

by rectal examination, ultrasonography, vaginoscopy, cytology evaluation, and uterine biopsy **(Garoussi *et al*., 2010)**. The pathological disorders in she-camel are due to uterine lesions which mostly inflammatory in nature. Moreover, metritis, uterine fibrosis, cysts, abscess and uterine neoplasms considered as the most acquired uterine lesions resulting in infertility in she-camel **(Shawky *et al*., 2004)**. The depth of inflammation of the uterine wall distinguishes uterine infection into metritis and endometritis **(Sheldon *et al*., 2006)**. Immunohistochemistry is an integral technique in many veterinary laboratories for diagnostic and research purposes **(Ramos-Vara 2005)**. Leucocytes are normal and variable component of the endometrial stromal cell population so the determination of excess leukocyte infiltrates which may be helpful in establishing a diagnosis of endometritis **(Disep *et al*., (2004)**. Leucocyte markers were characterized for clusters of differentiation (CD) as CD_{45}, CD_{20}, CD_{68}, CD_3 and CD_{56} **(Garner *et al.,* 2004 and Samatha *et al.,* 2013)**. The uterine defense mechanisms against microorganisms were maintained in several ways: anatomically, by their epithelium; chemically by glandular mucous secretions; immunologically, through the action of polymophonuclear inflammatory cells and humoral antibodies, but the degree of interaction is not clear **(Azawi, 2010 and Turner *et al.*, 2012).** The protective function of Acute Phase Proteins (APPs) against the damaging effects of enzymes formed during the inflammatory response that can lead to organ damage is also of significant importance. They are produced in the liver, and their concentration in the blood serum of cow increases, in response to uterine infection caused by microorganisms **(Sheldon *et al*., 2001 and Tothova *et al*., 2014).** Lysozyme present in most of body secretions such as saliva, milk and blood **(Mullan, 2001)**. It is a non-specific, disease resistance factor which hydrolyses the glycocidic bond between N-acetylemuramic acid and N- acetyle-D-glycosamine in bacterial cell walls **(Zou *et al.*, 1998)**. Nitric oxide (NO) occurs in many body systems and is produced by NO synthase (NOS) in both physiological and pathological states **(Gupta *et al*., 2010 and Zanetti *et al*., 2010).** Under normal conditions, endothelial and neuronal NOS constitutively produce NO in low amounts to participate in a variety of biological functions. While during inflammation, NO is produced by inducible NOS (iNOS) in large amounts **(Saxena *et al.*, 2000; Tripathi, 2007 and Li *et al.*, 2010)**. The increased NO produced by iNOS aids in the removal of pathogens, but can also be cytotoxic **(Tripathi, 2007)**. The higher levels of inflammatory cytokines TNF-α, IL-6 and IL-10 as well as acute phase proteins, Haptoglobin (Hp) and Serum Amyloid **A** (SAA) in the serum of cows may be associated with subclinical

inflammation of the uterus. Increased activity of immunocompetent cells, stimulated mainly in the uterus, but also in peripheral blood, could be the cause of an increase in the concentration of inflammatory mediators in the blood **(Brodzki *et al*., 2015).** Tissue-specific protein profile is determined by its function, structure, intensity of metabolism and usefulness that remains under sex hormonal control. Any disturbance in the general metabolism may be reflected in changes in both protein quantity and quality and some can be used as clinical markers of pathological conditions **(Kankofer *et al*., 2014)**. Protein molecules can be also altered by endogenous factors such as reactive oxygen species leading to peroxidative damage **(Kankofer, 2001).** This study aimed to evaluate the uterine infection of slaughtered she-camels through identification of bacterial agents colonizing the uterine environment, with observing the uterine histopathological picture, immunohistochemical and immunological view characterization of endometritis in order to find out which factors possibly influence the progress of endometritis.

MATERIAL AND METHODS:

I-Study Design:

A total of 100 uteri from slaughtered adult she -camels were collected for this study from Kerdassa, Nahia and El-Mounieb abattoirs. Ages of the animals were about (6-15) years old.

II-Samples Collection and Transportation:

Blood samples and uterus from each animal were collected. Then, they were transported on ice to the diagnostic laboratory within 1h.

III-Microbiological examination:

All uterine tissues were cultured on blood agar media, MacConkey's agar plates, Mannitol salt agar, Edward's medium and brain heart agar media then incubated at 37°C for 24–48 hrs. Suspect colonies were examined for colony morphology, Gram's characteristics and motility. Gram negative bacilli and Gram positive cocci were further subjected to catalase, oxidase and coagulase tests as well as standard biochemical tests **(Cowan and Steel, 1993 and Koneman *et al*., 2005)** to identify the isolates.

IV-Pathological examination:

A-Histopathological examination: It was applied according to **Suvarna *et al.* (2013).** The uterine tissue specimens were examined for any gross pathological abnormalities. Then, they were rapidly fixed in 10 % neutral buffered formalin for histological and immunohistochemical processing.Tissue sections were stained with Hematoxylin and Eosin, for studying the general structures.

B-Histochemical analysis:

Tissue sections were stained with Periodic acid Schiff technique (PAS) for mucopolysaccharides, Prussian blue stain for haemosiderin as well as Masson-trichrome stain was used for connective tissue detection and for the bacterial demonstration, used Giemsa stain according to **Suvarna *et al.* (2013).**

C-Immunohistochemistry: They were applied according to **Salem *et al.* (2012) and Rahmoun and Lieshchova (2014)** for clusters of differentiation (CD_3, CD_{20}, CD_{68} and CD_{138}) detection. The Primary and secondary antibodies were of murine origin and tissue sections were processed according to the manufacturer's directions (R and D systems Inc., HRP-AEC mouse kit system, Minneapolis, Minnesota, US). The staining of negative control was performed as before except that the primary antibodies were replaced with PBS, while the rest of procedures were maintained.

V-<u>Immunological examination:</u>

Blood samples were collected from slaughtered animals. Serum was obtained by centrifugation at 3000 rpm for 20 min and stored at -20°C.

A- Detection of lysozyme concentration:

Lysozyme assaying was done according to **Schultz (1987)**. The lysozyme diffuses through the agarose gel containing a suspension of Micrococcus lysodeikticus. A clear zone ring of lysis develops in the initially translucent agarose gel.

B-Measurement of serum nitric oxide (NO):

It was assessed according to the assay described by **Rajarman *et al.* (1998).** The test depends on that nitrite is a stable oxidation product of nitric oxide , which correlates with the amount of nitric oxide present in serum sample.

C-Determination of Haptoglobin (Hp) and Serum Amyloid A (SAA) concentrations: according to **El-Deeb, (2015),** the samples under this study were subjected to measurement of Hp and SAA using sandwich ELISA. Ready coated anti-bovine 96-well microtiter ELISA plates were applied (Sunredbio Co., Shangahi, China). The procedures were followed according to the instructions provided with the kits.

D-Analysis of protein profile of the tested uterine tissue samples using SDS-PAGE:

-Protein was purified from the uterine tissue samples according to **Dignam (1990).**

- Polyacrylamide gel electrophoresis, Combessies blue staining analysis of proteins was carried out by standard protocols **(Maniatis *et al.*, 1982).**the selected tissue samples with

endometritis where submitted to SDS-PAGE, and their protein patterns were compared with a database of normalized protein fingerprints derived from normal tissue samples.

E-Computer-aided analysis of the gels:

Images of the gels were captured using a sharp JX-330 flat-bed scanner and image analysis of the protein profiles was performed using Amersham Pharmacia Biotech Image master 2-D Elite software.

VI-Statistical Analysis:

The obtained results were statistically analyzed using Student test as described by **Petrie and Watson (1999)**.

RESULTS AND DISCUSSION:

Reproductive disorders in she-camel are rapidly becoming a major part of the veterinary care provided to the Camelidae. During the reproductive life of female, the uterus is exposed to the risks of infection, particularly at the time of breeding and following parturition **(Tibary, 2001).** Local immunity, phagocytosis and mechanical clearance by myometrial contractions are the major mechanisms used to clear uterine infection. Failures of these defense mechanisms leads to the establishment of a uterine infection with endometritis or metritis development, and usually occur when uterine resistance is diminished due to degenerative endometrial changes or repeated heavy infection **(Tibary and Anouassi, 1997).** Moreover, **Mshelia *et al.* (2013)** explained that decreasing the uterine immune status of the animals triggers bacterial adhesion, colonization and penetration of the uterine epithelium and/or release of bacterial toxins that lead to establishment of uterine diseases.

I-Microbiological examination:

Evidence implicating bacterial infections as causes of endometritis has been reported, and a variety of these bacterial species have been recovered from the uteri of infertile camelids **(Wernery and Kumar 1994; Tibary *et al.* 2006; Gwida *et al.* 2012 and Mshelia *et al.* 2014).** In this study, out of 100 examined uteri of camelids, bacteria were detected in 38 samples that found either in single or mixed infection with percentage (52.5%) and (47.5%), respectively. *S. aureus* was the most isolated bacteria as single infection with percentage of 26.2% followed by *E. coli* 15.8% then *S. pyogen* (10.5%) as mentioned in (Table 1). The results of uterine bacterial isolates observed in camels in the current study were concurred with the findings of **Yagoub (2005)**; **Nabih and Osman (2012) and Mshelia *et al.* (2013)** who reported *S. aureus, E. coli* and *Strept.* spp. as the main bacterial isolates from

several cases of uterine infections in she camels. While mixed infection in current study showed that *S. aureus* + *E.coli were* most prevalent as they represented 15.8% followed by *S. aureus* + *S. pyogen* with a percentage (10.5%) as mentioned in (Table 1).**Mshelia *et al.* (2013)** reported that *S. pyogenes* in dromedaries with endometritis, making these pathogens important causes of uterine disorders in camelids. Also the isolation of *S. aureus* and *E. coli* in large proportions in camelids with endometritis should be considered very important in the present study. *S. aureus*, *E. coli*, *Streptococcus* spp, *P.multocida* isolated from the uteri of cows with a history of metritis (**El-Azab *et al,* 1988).** In Egyptian she camels **Ali *et al.* (1987)** revealed that in cases of endometritis *E. coli and P.multocida* were common bacterial isolates .Also **Williams *et al.* (2005)** said that *P.multocida* was one of potential pathogens recovered from endometritis in cattle. The presence of *S. aureus*, *E. coli*, *S. pyogenes* and *P. multocida* in uteri of camelids were estimated in this study. These microbial agents are considered as important causes of uterine disorders in these livestock species and should be investigated during pre-breeding evaluation of susceptible females, as they could have far-reaching consequences on the reproductive performance if unchecked. Thus, it is suggested that female animals should routinely be evaluated bacteriologically and histopathologically against uterine disorders before their breeding seasons. The number of bacteria colonizing the uterus and the level of uterine immune response are important determinants of uterine infections, when the immune status is lowered; the pathogenic bacteria adhere to the endometrial mucosa, get internalized and penetrate the epithelium. Alternatively, the bacteria can also release toxins that cause uterine diseases **(Azawi 2008 and Singh *et al.* 2008).**

Table (1): Total number of isolates and percentages from uterus of she-camels.

Total isolates and percentages	No.	%
A-Single infection :		
1-S. aureus	**10**	**26.2**
2-E. coli	**6**	**15.8**
3-S. pyogen	**4**	**10.5**
Total single infection	**20**	**52.5**
B-Mixed infection:		
1-S. aureus + E. coli	**6**	**15.8**
2-S. aureus + S. pyogen	**4**	**10.5**
3-S.pyogen+ E. coli	**2**	**5.3**
4-E. coli +P. multocida	**2**	**5.3**
5- S. aureus + P. multocida	**2**	**5.3**
6- S. aureus + S. pyogen+ P. multocida	**2**	**5.3**
Total mixed infection	**18**	**47.5**
Total bacterial isolates	**38**	**100**

******Percent out of total positive uterus examined (n=38)**

II-<u>Pathological examination:</u>

Resident genital microbial agents are responsible for numerous diseases that directly or indirectly affect reproductive performance in Camelidae, and knowledge of this resident uterine microflora is relevant in understanding the pathological processes that could be observed **(Mshelia *et al*., 2013).** In this study, 38 uterine camel samples were found to be positive in bacterial cultures, subjected to histopathological examinations. According to both gross and microscopical findings, the recorded lesions of examined uterus of she camels were illustrated in (Table 2).

Table (2): Total numbers and percentages of pathological lesions she-camel uterus.

Pathological condition	No.	%
I-Acute endometritis: **1-Acute catarrhal endometritis**	**2**	**5.3**
2-Acute hemorrhagic endometritis	**4**	**10.5**
3-Acute suppurative endometritis	**3**	**7.9**
II-Sub-acute endometritis	**3**	**7.9**
III-Chronic endometritis: **1-Chronic catarrhal endometritis**	**10**	**26.3**
2-Chronic cystic endometritis	**16**	**42.1**
Total uterine lesions	**38**	**100**

******Percent out of total positive uterus examined (n=38).**

In the current study, endometritis represented 38% that were nearly similar to results of **Waheed *et al*., (2009)** who reported endometritis in a percentage of 24%. Among these inflammatory conditions, chronic endometritis was the most common recorded endometritis type (68.4%) followed by acute endometritis (23.7 %) then sub-acute endometritis (7.9 %). These results were in agreement with **Abd EL-Aal (1998) and EL Deeb (1995) and** disagree with many authors **(Hegazy *et al.*, 1998 and Moustafa *et al*., 2004)** who mentioned that; acute catarrhal endometritis was the most frequently uterine lesion in she camels. The variation in intensity of the uterine inflammatory changes was attributed to the host resistance, environment and virulence of microorganisms **(Abd El-Wahab, 1991). Tibary and Anoussi (2000)** considered that untreated uterine infections can lead to irreversible changes infertility.

I-Acute endometritis:

In this study, all cases of acute endometritis showed sub-epithelial inflammatory cell infiltrations predominantly neutrophils with few lymphocytes and macrophage as well as in the Lumina of dilated uterine glands that completely replaced necrotic glands. There was severe edema in the mucosa and submucosa associated with vasculitis.

1-Acute catarrhal endometritis: - It was recorded in 2 cases (5.3 %); *S. aureus* (1 case) as well as *S. Pyogen* (1 case) was isolated as single infections. Grossly, the uterus was enlarged and their mucosa was congested and edematous in addition to presence of turbid mucous exudate on the endometrium was also seen. Microscopically, they characterized by cellular infiltrations associated with alternative areas of desquamation and hyperplasia of endometrial epithelium as well as degenerated glands were seen Fig. **(**1). Glandular goblet cell hyperplasia also noticed and confirmed with (PAS) stain that gave strong positive reaction Fig. (2).

2-Acute hemorrhagic endometritis: Our results indicated 4 cases (10.5 %) in this type. *E. coli* (2 cases) and *S. aureus* + *S. pyogen*+ *P. multocida* (2 cases) were isolated as single and mixed infections, respectively. Macroscopically, the uterus was enlarged and the mucosa was severely congested, multiple areas of petechial hemorrhages and blood tinged thick exudates in the uterine lumen were seen. The histopathological examination revealed marked endometrial necrosis together with focal epithelial ulceration and the presence of multiple hemorrhagic areas that infiltrated with inflammatory cells Fig. (3 and 4). Lamina propria showed presence of haemosidrin pigments, which were either free or within macrophages that confirmed with Prussian blue stain.

3-Acute suppurative endometritis: Three cases (7.9 %) were recorded and *S. aureus* (2 cases) as single infection as well as *S. aureus* + *S. pyogen* (1case). Macroscopically, the uterine mucosa was congested and covered with thick creamy whitish purulent exudates. Microscopically, there was marked sub-epithelial and periglandular cellular infiltrations mainly neutrophils as well as severe endometrial epithelial necrosis, desquamations and sever vasculitis were noticed Fig. (5). The present study demonstrated that both macroscopic and microscopic changes related to acute uterine infections in she-camels similar to that observed in the previous reports **(Gehan El-Sakkar *et al*., 2008; Singh *et al*., 2008** and **Mshelia *et al*., 2013).** The uterine blood vessels changes noticed in this survey may lead to ischemia and subsequently bad impact on the uterine functions which agree with observation of **Wajid (2015).** In fact, our data showed that, the bacterial infection disrupts endometrial structure and function, as mentioned previously by **Sheldon *et al*. (2009)**. Moreover, **Dolatkhah *et al*. (2013)** indicated that the endometrial cells could secrete cytokines and chemokines for PMNs and macrophages attraction to eliminate the bacteria. We noted that there were uterine lesions associated with the presence of *S. aureus*, *E. coli*, and *S. pyogenes* and that was mimic to results of **Mshelia *et al.* (2013).** On the other side, **Yilmaz *et al*., 2012** mentioned that *E. coli, S. aureus* and *Bacillus spp*. as mixed isolates related to the mild type of endometritis and this disagree with our results so *E. coli* is needed to damage the endometrium enabling absorption of endo-toxins and a damaged epithelium is usually required to establish infection as it can suppress the defense mechanism of the uterus itself and facilitate other organisms to participative in the infection **(Dar *et al*., 2016)**. Also, **Martins *et al*. (2016)** described acute suppurative endometritis as a result of *E. coli.* Four cases of acute haemorrhagic endometritis (10.5 %) were observed in the present study. This result disagreed with those of **Tibary (2001) and Moustafa *et al.* (2004)** who recorded it with an incidence of 0.4 %. In this respect, **Abdullah *et al*. (2014) and Madboli and Eldebaky (2016)** described hemorrhagic and suppurative endometritis due to *P. multocida* as it could be reached to the reproductive system through infiltrated macrophages and able to induce prominent histopathological changes in uterus which reduce the reproductive performance of animals. The observed hemorrhages and odema were resembled to the results of **Dagleish *et al*. (2010) and Rhyaf (2010) and Taylor *et al*. (2010)** who attributed them to the cytokines released from the activated macrophages and PMNs under the effect of extracellularly injury and lipopolysaccharide toxins of *P. multocida*. In this respect, **Ibrahim *et al.* (2016)** related the pathogenicity of *P. multocida*

to their irreversible pathological changes in the reproductive organs which might have a direct proportional effect on the reproductive ability of the animals that survived *P. multocida* disease, especially the carrier ones.

II- Sub-acute endometritis:

This inflammatory type was found in 3 she-camels (7.9 %). *S. Pyogen* (1case) and *E. coli* (1case) as well as *S. aureus* + *E. coli* (1case) as single and mixed infections. Grossly, the examined uteri were apparently normal. Microscopically, it characterized by subepithelial leucocytic infiltration associated with hypertrophy of the blood vessels and oedema.

III-Chronic endometritis:

In the current study, *S. aureus, S. Pyogen, E. coli and P. multocida* were isolated as single and/or mixed infection in this type. Grossly, the endometrium was thickened and corrugated, dry and rough. The microscopic examination of these cases revealed marked local and/or diffuse inflammatory cell infiltrations mainly lymphocytes, plasma cells and macrophages associated with connective tissue proliferation. Multiple polypoid like projections or hyperplasia of endometrial mucosa were also seen. Moreover, vacuolar degeneration and inflammatory cell exocytosis of the lining and glandular epithelium was observed.

1-Chronic catarrhal endometritis:

There were 10 cases (4 cases *S. aureus*, 1 case *E. coli, and 2 cases S. pyogen* as single infection *and* 3 mixed infection cases) of this type with a percentage of 26.3 %. Microscopically, they revealed inflammatory cell infiltrations with connective tissue proliferation and hypertrophy in the vascular wall Fig. (6).

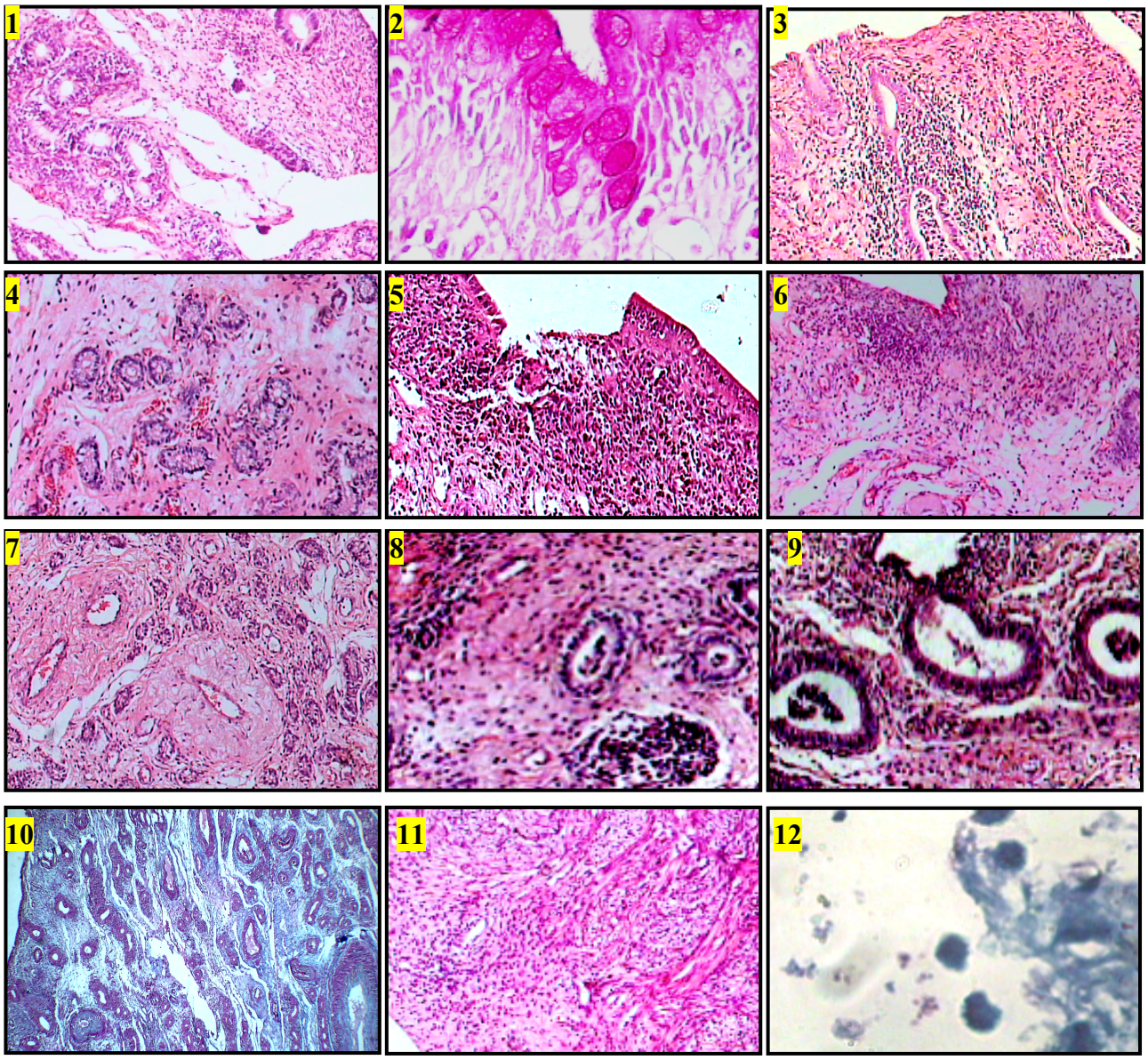
2-Chronic cystic endometritis:

Sixteen cases (3 *S. aureus* cases and 2 *E. coli* cases as well as 11mixed infection cases) of them were noticed (42.1 %). They characterized by irreversible changes including atrophy and necrosis of the endometrial glands Fig. (7). Endometrial gland nests, associated with cystic dilation or lymphatic cysts are observed Fig. (8 and 9). These degenerative changes are mainly due to the presence of periglandular or perivascular fibrosis which demonstrated with Masson-trichrome stain Fig. (10). Out of 26 cases of chronic endometritis, there was 8 cases showed metritis in which the inflammatory cell infiltrations extended to the muscular layer and serosa, were observed Fig. (11).

Similar histopathological finding were described previously **(Tibary and Anouassi, 2001; Nourani *et al.* 2003;Moustafa *et al*., 2004; Mshelia *et al*., 2012 and Wajid, 2015).** The indicated chronic endometritis with permanent fibrosis which came in accordance with **Fetaih (1992),** who considered uterine fibrosis of she camels, is outcome of metritis resolution. The noticeable degenerative changes are mainly due to the presence of periglandular or perivascular fibrosis as mentioned before by **Tibary (2001) and Ghoneim *et al.* (2013).** Reinforced the previous findings of **Dolatkhah *et al*. (2013)** who mentioned that, the staining density and glands cellular solidarity decreased and percent of fibrotic regions increased markedly in endometric cows. Moreover, periglandular fibrosis might reduce the she camel's fertility as a result of loss of the secretory activity of such glands which produce unfavorable site for implantation **(Mahdy, 1988).** We noted endometrial hyperplasia which might be as a result of dense infiltration of inflammatory cells and this agreed with **Rhyaf (2010).** In the present study, PAS stain was done for detection of goblet cell hyperplasia which came in accordance with **Sharma *et al.* (2016**). The decrease in the amount of glycogen reflects the gradual decline in the activity of these tissues which reflects a degenerative process in the uterine endometrium. In this work we could demonstrate the isolated bacteria in the endometrial tissue sections with Giemsa stain Fig. (12). Studies on the cellular populations of healthy and diseased camel uterus are very scarce. Due to the lack of camel specific primary antibodies, anti-mouse and anti-rabbit were used and that was in accordance with previous studies in different organs **(Zidan *et al*., 2000; Salem *et al*., 2012 and Al-Ashqar *et al.,* 2015).** Immunohistochemistry is an integral technique in many veterinary laboratories for diagnostic and research purposes **(Ramos-Vara 2005).** Endometritis is mediated by presence of T and B lymphocytes and plasma cells **(Samatha *et al*., 2013).** Demonstration of immune cells in endometrial tissues by immunohistochemistry revealed the distribution and type of immune cell in chronic endometritis cases in the present study. In control specimens, endometrial leukocytes were composed of T lymphocytes (CD_3) and macrophages (CD_{68}) positive cells which localized sub-epithelium as well as periglandular and perivascular aggregates with an absence of B lymphocytes (CD_{20+}) and plasma cells (CD_{138+}). In addition to, the immunohistochemical studies of chronic endometritis cases revealed prominent increase in CD_{3+} Fig. (13), CD_{20+} Fig. (14) and CD_{68+} Fig. (15) with the severity of inflammation associated with presence of CD_{138+} Fig. (16). Our observations were in accordance with **Tawfik *et al*., (1996) and Samatha *et al*., 2013** who observed increase in CD_{20} and CD_3

positive cells up to 50 folds and 3 fold respectively in endometritis cases. Also, **Zidan and Pabst (2002)** can detect CD_3 positive lymphocytes in camels. On the other side, no difference in number of T lymphocytes in normal and endometritis cases was reported by **Disep *et al.*, (2004). Tawfik *et al.* (1996)** mentioned that there were two populations of lymphocytes (T helper and B lymphocytes) responsible for antibody response and their proliferation may result from the antigenic stimulation exerted by the different organisms. IL_2 secreted by T-cells after antigenic stimulation then followed by release of cytokines by T_2 cells and that induces humoral immunity by inducing proliferation of local and regional lymph nodes and cause increase in B cells and T cells. B cells proliferate and differentiate to produce plasma cell and then antibodies **(Azawi, 2008)**. The increased number of immune cells in chronic endometritis has the ability to produce variety of cytokines and growth factors that have harmful effect on pregnancy leading to abortion and infertility **(Bondurant, 1991).** Furthermore, **Tawfik *et al.* (1996)** stated the plasma cells remain the hallmark for the diagnosis of chronic endometritis. The diagnosis of chronic endometritis depends upon detection of plasma cells within inflammatory infiltrate in endometrium **(Samatha *et al.*, 2013).** The importance of usage CD $_{138}$ is proved to get rid the wrong diagnosis, because of it's specifically which stain only plasma cells, and can get rid of any possibility for false H&E positive results in the diagnosis of chronic endometritis **(Patrick *et al.*, 2010).** The detected CD $_{138}$ in this study was similar to that of **Illene *et al.* (2001); Naji (2012) and Samatha *et al.* (2013)**.

FIGURES



(Fig.1): She-camel endometrium showing acute catarrhal endometritis with prominent lymphocytic cellular infiltrations, degenerated glands and edema **(H&E, x100).**

(Fig.2): She-camel endometrium showing strong positive reaction of PAS **(PAS stain, x400).**

(Fig.3): She-camel endometrium showing acute hemorrhagic endometritis characterized by epithelium ulceration, multiple hemorrhagic areas and dense inflammatory cells infiltrations **(H&E, x100).**

(Fig.4): She-camel endometrium showing acute hemorrhagic endometritis characterized by periglandular hemorrhagic areas and inflammatory cells infiltrations with vascular congestion **(H&E, x100).**

(Fig.5): She-camel endometrium showing acute suppurative endometritis characterized by marked subepithelial cellular infiltrations, epithelial necrosis, desquamations and vasculitis **(H&E, x100).**

(Fig.6): She-camel endometrium showing chronic catarrhal endometritis with massive inflammatory cell infiltrations, glandular atrophy and congestion **(H&E, x100).**

(Fig.7): She-camel endometrium showing chronic cystic endometritis with prominent glandular atrophy, periglandular and fibrosis and hypertrophy in the vascular wall **(H&E, x100).**

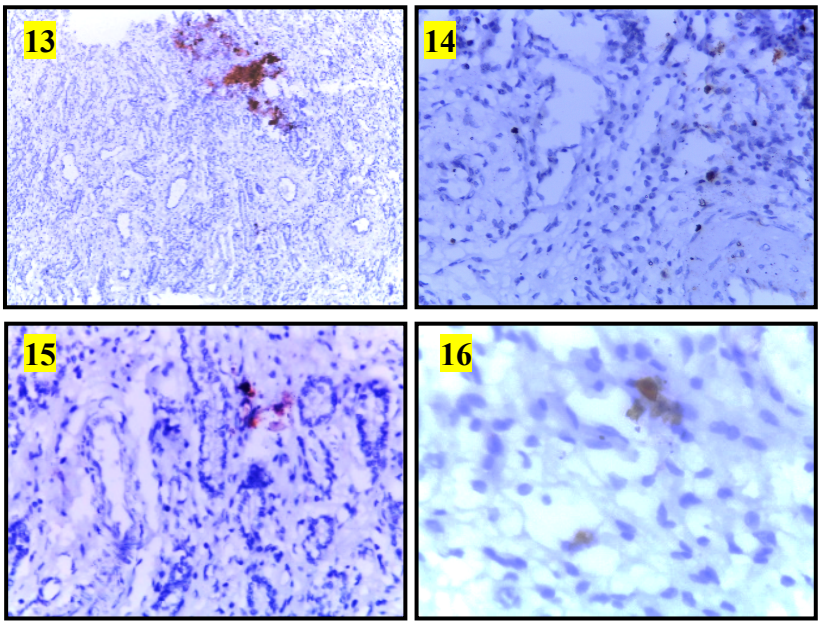
(Fig. 8): She-camel endometrium showing chronic cystic endometritis characterized by glandular atrophy and necrosis as well as periglandular and perivascular fibrosis **(H&E,x400).**

(Fig.9): She camel endometrium showing chronic cystic endometritis characterized by cystic dilatation and necrosis as well as periglandular and perivascular fibrosis **(H&E, x400).**

(Fig.10): She-camel endometrium showing diffuse periglandular and perivascular fibrosis **(Masson-Trichrome stain, x40).**

(Fig.11): She-camel endometrium showing metritis with inflammatory cell infiltrations extended to the muscular layer and serosa **(H&E, x100)**

(Fig.12): She-camel endometrium showing *S. aureus* and bipolar *P. multocida* **(Giemsa stain, x1000).**



(Fig.13): She-camel endometrial stroma immunostained showing scattered subepithelial and periglandular positive immunostaining for CD_3 **(T lymphocytes) (x40).**

(Fig.14): She-camel endometrial stroma immunostained showing scattered perivascular and periglandular positive immunostaining for CD_{20} **(B lymphocytes) (x100).**

(Fig.15): She-camel endometrial stroma immunostained showing periglandular positive immunostaining for CD_{68} **(macrophages) (x100).**

(Fig.16): She-camel endometrial stroma immunostained showing positive immunostaining for CD138 **(plasma cell) (x100).**

II-Immunological examination:

The number of bacteria colonizing the uterus and the level of uterine immune response are important determinants of uterine infections **(Mshelia *et al.*, 2014).** The innate immune system in endometrium, as elsewhere, must protect against infection while signaling the presence of a pathogen to the acquired immune system in the event that infection does occur **(Anne *et al.*, 2003).** Respectively, since APPs are sensitive innate immune molecules, they are useful for early detection of inflammation in bovines and believed to be better discriminators than routine hematological parameters. Therefore, the possibility of using APPs as a diagnostic and prognostic marker of inflammation in major bovine health disorders including postpartum uterine infection has been explored by many workers **(Manimaran *et al.*, 2016).** In the running experiment, we measured the levels of some selected immunological parameters as biomarkers for the immune status of she-camels with sub-acute, acute endometritis and normal she-camels. The results were shown in (Table 2).

Table (3): Results of some immunological parameters in serum of examined she-camels.

Immunological parameter	Normal (Mean ±S.E)	Sub-acute	Acute
Nitric Oxide	**0.2±0.03**	**1.2±0.6**	**2.6±0.8**
Haptoglobin	**0.35±0.1**	**1.6±0.8**	**4.2±1.5**
Serum Amyloid A	**0.8±0.3**	**1.8±0.7**	**3.7±1.2**
Lysozyme	**0.5±0.2**	**0.6±0.1**	**0.9±0.3**

S.E = Standerd Error

The previous table showed that the NO concentration was significantly higher in serum samples of she-camels with acute endometritis than that of sub-acute endometritis, and in the sub-acute cases when compared with the samples of apparently normal she-camels. These results agreed with that of **MY *et al.* (2003) and Rocha *et al.* (2015),** who detected greater amounts of NO and NOS in the endometrial tissues of women with endometriosis, implying a possible role for NO in the pathogenesis of endometriosis. Meanwhile, **Li *et al.* (2010) and Song *et al.* (2015)** recorded that, cows with clinical or subclinical endometritis showed higher concentrations of NO in both plasma and uterine secretions when compared with normal cows and the highest concentrations of it were found in clinical endometritis cows. Regarding the Hp and SAA, the current study revealed a significant elevation in their levels, in the serum

samples of she-camels with sub-acute and acute endometritis cases when compared with the samples of apparently normal she-camels. These results were in the same line with the results conducted by **Brodzki *et al.* (2015)**, who clearly ascertained that, the levels of haptoglobin (Hp) was significantly higher both in serum and uterine washings in cows with endometritis. They added that, the evaluation of the levels of cytokines and Hp in serum and uterine washings considered to be an important diagnostic marker of inflammation of the uterus in cows. Thus, **Tothova *et al.* (2014)** found that various APPs, haptoglobin (Hp) and serum amyloid A (SAA) are the primary positive biomarkers of various types of diseases in cattle. Meanwhile, **Brodzki *et al.* (2015)** confirmed that, the level of SAA was significantly higher in the serum of cows with endometritis compared to healthy animals. Our investigation also cleared that, the rise in the levels of lysozyme were not statistically significant between the samples of the tested groups with various forms of endometritis. And these results were nearly similar to that reported by **Katila *et al.* (1990),** who conducted a study to determine differences in the inflammatory response following bacterial challenge between normal mares and mares with chronic endometritis; they concluded that factors other than neutrophil numbers, lysozyme and alkaline phosphatase activity account for the inability of the mare to eliminate uterine infections.

Table (4): The effect of the isolated microorganisms on the immunological parameters.

	Immunological parameters			
Bacterial isolates	**Lysozyme**	**N.O**	**Hp**	**SAA**
S. aureus	**0.5±0.1**	**4.1±1.5**	**3.6±1.3**	**3.1±1.4**
E. coli	**0.9±0.2**	**2.2±0.9**	**4.7±1.2**	**3.9±1.4**
S. pyogen	**0.7±0.1**	**2.6±0.8**	**3.1±0.9**	**2.7±1.1**

One of the objectives of this work was to study the effect of the isolated microorganism on the levels of Lysozyme, NO, Hp, and SAA in the examined samples. The significant increase in NO concentration obtained from samples affected with *S. aureus* was obvious when compared with samples affected with other isolated microorganisms (Table 4). Similar results were obtained by **Attia *et al.* (2003) and Komine *et al.* (2004),** who found that NO concentration in *S. aureus* infected mammary glands, were significantly higher than its concentration in those infected with *CNS*. While, **Sorge *et al.* (2013)** reported that**,** *S. aureus* is among the few bacterial species that express nitric-oxide synthase (NOS) and thus can

catalyze NO production from L-arginine. Thus, **Carey *et al.* (2015)** study showed that a *S. aureus* product elicits an NO-mediated innate defense response in human upper airway epithelium. And **Melinda *et al.* (2016)** found that**, e**xposure of *S. aureus* to NO typically results in growth inhibition and induction of stress regulons. Also, (Table 4) showed that, the Hp concentration in *E. coli* infected uterine samples was significantly higher than its concentration in samples infected with other isolated microorganisms. This might run in parallel with the results obtained by **Suojala *et al.* (2008) and Wenz *et al.* (2010)** who noticed that, the concentration of Hp were the highest in *E.coli*-induced mastitis compared with mastitis caused by environmental *streptococci* or *CNS* indicating the sever inflammation induced by *E. coli*. At the same time, a field study applied by **Pyörälä *et al.* (2011)** summarized that, the concentration of Hp in milk vary depending on which pathogens were isolated; its concentrations were the highest in naturally acquired mastitis caused by *E.coli*, significantly lower in that caused by *streptococci* or *S.aureus* and it was the lowest in mastitis caused by *CNS*. Lately, **Mircheva *et al.* (2009)** conducted a study to evaluate the changes in the blood concentrations of haptoglobin (Hp), ceruloplasmin (Cp) and fibrinogen (Fb) during experimentally induced *E. coli* infection in weaning rabbits. Hp concentrations dramatically increased after *E. coli* inoculation since 24th hours, reached maximal values on day 7 (multiplied by a factor 9) and remained significantly elevated compared to basal values until the 30th day. The previous results showed in (Table 4) revealed that the SAA concentration in *E. coli* infected samples was significantly higher than its concentration in samples infected with other isolated microorganisms. These results were explained by **Ranjeeta *et al.* (2005) and Chandrabala *et al.*(2016),** who observed that SAA binding to a surprisingly large number of Gram-negative bacteria, including *E. coli*, *Salmonella typhimurium, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae,* and *Pseud. aeruginosa,* through outer membrane protein A (OmpA) family members. The binding was found to be of high affinity and rapid. Importantly, this binding was not inhibited by high density lipoprotein with which SAA is normally complexed in serum. The one-dimensional SDS-PAGE of uterine tissue samples revealed protein profiles containing (4-8) discrete bands with molecular weights of (37.74 -64.71) kDa. Fig. (17). We used the UPGMA clustering dendrogram analysis to compare the protein fingerprints of she-camels that showed sub-acute endometritis and that of the samples of apparently normal she-camels. Fig.(18). The present dendrogram illustrated that there were weak similarities in the protein fingerprints between samples from she-camels

infected with sub-acute endometritis and that of apparently normal she-camels ranging from (0.25 - 0.33). Based on these notable weak similarities, we can conclude that, there were great differences in the protein profile of she-camels with sub-acute endometritis and that of apparently normal samples. These results may lead us to suggest that the present technique may be used in differentiation between animals with and without endometritis. The same suggestion was accepted by **Thiago and Rodrigo (2012),** who used the dendrogram in illustrating the similarities among the bacterial profiles in different cows with different health status along postpartum period (DPP) and **Camilla *et al.* (2013)**, who used the dendrogram in the differentiating between three groups of mares infected with endometritis.

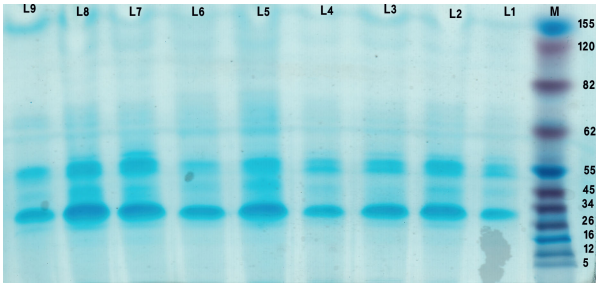


Fig (17): SDS-PAGE electrophoresis of apparently normal she-camels tissues and tissues of she-camels with sub-acute endometritis.

M: protein marker.

L1 and L9: Tissues of apparently normal she-camels.

L2 –L8: Tissues of she-camels with sub-acute endometritis**.**

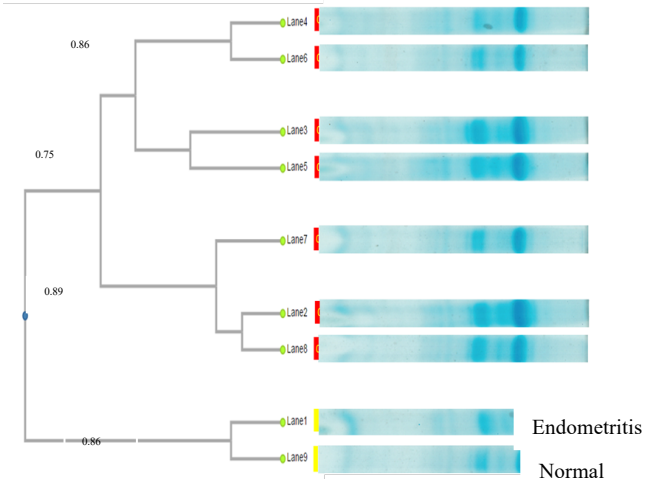


Fig (18): Dendrogram analysis of the uterine tissues protein bands from she-camels with sub-acute endometritis and samples of apparently normal she-camels.

CONCLUSION

1-The present study concluded that the high incidence of endometritis in she- camels may reflect the importance of isolated microorganisms. *S. aureus* and *E. coli* were the major bacterial pathogens colonizing the uterine environment in these species, commonly associated with inflammatory and degenerative changes.

2-The endometrial histopathology in she-camels might be useful and accurate procedures for detecting severity of endometritis and to prevent its progress to chronic with permanent changes. The immunohistochemical characterization of endometrial leucocytes may be helpful in establishing a diagnosis of endometritis in equivocal cases.

3- We can assume that the assessment of acute phase proteins levels can be an important diagnostic indicator of sub-acute endometritis in she-camels. Dendrogram analysis could be used to compare the protein fingerprints of she-camels showing sub-acute endometritis and that of the samples of apparently normal she-camels.

4-The incidence of endometritis can be reduced only if the chance of contamination of the uterus is minimized by implementing strict hygiene practices during breeding and parturition. Females should be examined for presence of uterine infection before mating to avoid contamination spread.

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