Summary

The present study was designed to identify the uncultivated and cultivated pathogens in vaginitis, in addition, quantify the occurrence of these pathogens in vagina by using conventional PCR and Real-Time PCR ,and find the relationship of some proinflammatory Bio-markers production with infectious vaginitis . A total of 250 vaginal swabs and punch biopsies from Cervix (200 with infectious vaginitis and 50 Non-infectious vaginitis) were collected from three Maternity and Pediatrics Teaching Hospitals(Ibn Al-Atheer , Al-Khansa′a and Al-Batool)in Al-Mosul city from December 2013 till end June 2014.

A smear of vaginal discharge from a bacterial vaginosis (BV) patients showed typically clue cells with mixed flora consisting of large numbers of small gram-negative rods and gram-variable rods and coccobacilli. Amsel's clinical criteria for bacterial vaginosis, which includes vaginal pH, whiff test, clue cells, and appearance of the vaginal discharge were recorded . Nugent score examinations results showed the presence of the relative amount of three bacterial morphotypes: Large Gram-positive rods (*Lactobacillus* species), small Gram-negative and Gram-variable rods (*Gardnerella vaginalis* and *Bacteroides* species) and curved rods (*Mobiluncus* species).

The DNA of all specimens was extracted and purified using genomic DNA purification kit. The results were detected by electrophoresis on 1% agarose gel and exposed to U.V light in which the DNA appeared as compact bands. The results of primers amplification of target genes (16SrRNA) and (18SrDNA) of all the studied extracted DNA specimens conventional PCR were 1046bp for *Atopobium vaginae* ; 299bp for *Bacteroides fragilis ;* 1107bp for *Gardnerella vaginalis* ; 1044bp for *Megasphaera* sp. ; 478bp for *Mobiluncus mulieris* ; 137bp

for *Mycoplasma hominis* ; 131bp for *Lactobacilus acidophilus* ; 114bp for *Candida albicans ;* 74bp for *Trichomonas vaginalis* .

The *lactobacillus acidophilus* in the present study were used in quantification PCR as a reference standard isolates for DNA serial dilution preparation for DNA copy number and standard curve plotting. The amplification plot of genomic DNA template concentration (cn) (1×10^8, 1×10^6, 1×10^4, 1×10^2 copy number) has shown clear differences in values of threshold cycle numbers (C_T).

The present study revealed that microbial load (mean DNA copy number = mRNA transcript level) of vaginitis ranged from 9.62×10^2 to 4.06×10^7 of vaginal swabs. the quantification PCR results showed that the *Candida albicans* and *Bacteriod fragalis* constituted 12/15(80%) ; *Lactobacillus acedophilus* and *Atopobium vaginae* 10/15(66.6%) , then *Mycoplasma hominis* 9/15(60%) , *Trichomonas vaginalis* 7/15(46.6%) , *Megasphaera* sp. 5/15(33.3%) and *Mobiluncus* sp. 4/15(30%) .

The study showed the presence of inflammatory cell infiltrate cytotoxic T cells (CD8), macrophages (CD68), perforin, T helper 1 cells (Tbet), T helper 2 cells (Gata 3) and T regulatory cells (Foxp3) and T helper 17 cells (Ih17) positive cells in patients with infectious vaginitis using immunohistochemical staining and compared with control group (non-infectious vaginitis).The scoring levels of CD8 and perforin positive cells were significantly higher in patients with vaginitis (mean 2.4± SD0.5) more than in patients with non-vaginitis (1.7±0.5) (p=0.007). Similarly, scoring levels of perforin was also significantly higher in patients with infectious vaginitis (2.4± SD 0.5) than in patients with non-infectious vaginitis (1.3±0.8) (p=0.003) , CD8 and perforin positive cells were present in the uterus section from Cervix of patients with vaginitis in all patients 9/9 (100%) in whom 4/9 (44%) had a mean score of > +3 and 5/9 (46%) had a mean score of >+2 for both CD8 & Perforin

. However, the patients control (non-vaginitis) showed also CD8 and perforin positive cells but at lesser extent in whom 4/6 (67 %) had a mean score of >+2 and 2/6 (33.%) had a mean score of >+1 for CD8 while 5/6 (83 %) had a mean score of >+1, 1/6 (16 %) had a mean score of >+3 for perforin. While the bio-markers (CD68, Foxp3, Gata3, Tbet, Th17) were are non-significant in both infectious and Non-infectious vaginitis.