

## Original research article

Prevalence of genital tract infection with *Entamoeba gingivalis* among copper T 380A intrauterine device users in EgyptAshraf A. Foda<sup>a,\*</sup>, Mohamed M. El-Malky<sup>b</sup><sup>a</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt<sup>b</sup>Department of Parasitology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

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## Abstract

**Background:** This study was performed to study the prevalence and potential pathogenicity of *E. gingivalis* in the genital tracts of intrauterine contraceptive device (IUD) users.

**Study Design:** A prospective study conducted at the Obstetrics and Gynecology Department and Fertility Care Unit, Mansoura University Hospital, Egypt. The study was carried out on 87 IUD users and 87 nonusers. The copper T 380A IUD was removed from each woman and washed with phosphate-buffered saline (PBS) pH 7.4; the IUD wash was centrifuged. The sediment was resuspended in 2 ml PBS and divided into two portions. One portion was used for preparation of direct and iron hematoxylin-stained smears. Direct smears and stained smears were examined for detailed morphology. The second portion of the sediment was used for DNA extraction and subsequent PCR amplification targeting the small subunit ribosomal RNA of *E. gingivalis*.

**Results:** The parasite was found in 12.64% of IUD users and in 6.9% of non users ( $p>.3$ ). It was found that 90.9% of those harboring *E. gingivalis* in their genital tract had the parasite in their oral cavity. The percentage of genital infection in IUD users increased with low level of education, rural areas, insertion in primary health-care center and among those not washing hands before checking the strings. In the infected cases, vaginal discharge was more common (81.8%) than in noninfected cases (32.9%), such difference was statistically significant ( $p<.05$ ). Also, excessive vaginal discharge is more common than backache and menorrhagia in the infected cases.

**Conclusion:** Higher incidence of *E. gingivalis* infection in IUD users is related to oral cavity infection, residence, the facility where they inserted their IUD and washing hands attitude before checking the strings. We recommend treatment of gingival infection, proper counseling and medical education on oral and genital tract hygiene for IUD users.

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**Keywords:** Intrauterine device; Copper T 380A; *E. gingivalis*; Genital tract infection

## 1. Introduction

*Entamoeba gingivalis* belongs to the Rhizopodia class, Entamoebidae family and suborder Tubulinae. It was the first amoeba to be described in humans. This parasite inhabits the area around teeth and gums, even in the space between teeth especially in decayed teeth cavities [1]. In some cases, it has been isolated from tonsil crypts and tonsil tissue sections [1–3]. There is also one report of the presence of *E. gingivalis* in sputum smears [4].

*E. gingivalis* is considered to be a scavenger, as it is found in the healthy oral cavity [5]. It may contribute to periodontal diseases [6,7], and it was detected using computed tomography-guided fine-needle aspiration in a pulmonary abscess, which resolved after treatment with clindamycin followed by penicillin [8].

Morphologically, this protozoan is similar to *E. histolytica*, and diagnosis requires enough attention so *E. gingivalis* could be differentiated from it. Like *E. histolytica*, the karyosome is centrally located in the nucleus. This parasite is found only in trophozoite form, which varies from 5 to 35  $\mu\text{m}$  [1] and has the ability to phagocytize not only bacteria and other organisms but also leukocytes [9]. On permanent stained smears, nuclear fragments of leukocytes can be seen within the food vacuoles, this fact helps to differentiate it from other amoebae since *E. gingivalis* is the only species

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that ingest fragments of leukocytes [9]. Among different ways of transmission are close contact, contaminated food, dishes, mouth droplets and kissing [10].

Prevalence rate of infection to *E. gingivalis* is related to age and sex. The infection is rarely found before the age of 20 years with peak incidence at the age of 21–30 years [11]. In women, since dental and mouth care is more frequent than in men, the infection to *E. gingivalis* is less prevalent than in men [11].

The diagnosis of *E. gingivalis* is based on riboprinting, lack of cyst, morphology of trophozoite with failure to stain with anti-*Entamoeba histolytica* serum. It is best stained with trichrome vitelli staining method using bovine fixative [10,12].

The vagina and uterus are regarded as suitable growth media for *E. gingivalis*, and the existence of this parasite in the genital tract warrants wider recognition among IUD users [12].

Intrauterine contraceptive device (IUD) is the contraceptive method chosen in Egypt by about 17% of married women of reproductive age [13]. Thus, it is likely that genital tract infection with *E. gingivalis* may affect a lot of women in Egypt.

This work has been conducted to study the prevalence and potential pathogenicity of *E. gingivalis* in the genital tracts of copper T 380A IUD users.

## 2. Materials and methods

This study was carried out on 87 cases using the copper T 380A IUD as a contraceptive method and on 87 nonusers (as a control group) attending the Gynecology and Obstetrics Outpatients' Clinic of Mansoura University Hospital, Egypt, during the period from April 2009 to October 2010. These cases were chosen during their attendance to the outpatients' clinic for IUD removal after counseling. The inclusion criteria were cases with inserted copper T 380A, the duration of IUD use from 3 to 5 years and no associated pelvic inflammatory disease (PID) or tumors. The exclusion criteria were the use other types of IUDs, PID and IUD use for less than 3 years. These cases asked for IUD removal either to change the method of contraception or to get pregnant or to treat any associated complaint. The duration of use of IUD ranged from 36 to 56 months.

A full history was taken from each patient including residency, education, health center approached for IUD insertion, hygienic practices before checking the strings of the IUD and any complaints.

A written consent has been obtained from every patient enrolled in this study. The study was approved by the ethical committee of Mansoura Faculty of Medicine.

The IUD was removed from each woman on her request to change the method of contraception, to get pregnant or to treat any associated complaint. The removed IUD was washed with phosphate-buffered saline (PBS) pH 7.4, and

the IUD wash was centrifuged at 500×g for 10 min. The sediment was resuspended in 2 ml PBS and divided into two portions. One portion was used for preparation of direct and iron hematoxylin-stained smears. Direct smears were examined microscopically for any motility. Stained smears were examined for detailed morphology according to Spencer–Monroe method [14]. *E. gingivalis* was considered present if trophozoite motility or morphology was observed.

The second portion of the sediment was used for DNA extraction and subsequent PCR amplification targeting the small subunit ribosomal RNA (SrRNA) of *E. gingivalis* according to the method described by Kikuta et al. [15]. DNA from loop sediment was extracted by phenol chloroform. In brief, sediment was resuspended in 10 ml PBS and mixed with an equal volume of phenol and centrifuged for 5 min at 1200×g. The aqueous phase was transferred to a new tube, mixed with equal volumes of phenol chloroform (1:1) and centrifuged 5 min at 3500 rpm. Again, the aqueous phase was transferred to a new tube, mixed with an equal volume of chloroform and centrifuged 5 min at 3500 rpm. DNA was precipitated by adding 1/10 volume of 3 M sodium acetate and 1 volume of 99% ethanol. After centrifugation for 1 h at 14,000×g, the supernatant was discarded. To remove residual salt, the pellet was washed with 1 ml of 70% ethanol and centrifuged 20 min at 10,000 rpm. Supernatant was discharged. The DNA pellet was air-dried and dissolved in 20 ml of water and stored at –20°C until use. Primers used were forward, 5'-GAATAGGCGCG-CATTTCTGAACAGG-3' and reverse 5'-TCCCACTAG-TAAGGTACTACTC-3'. PCR condition, 30 cycles, each cycle consisted of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, with an extension of 4 min at 72°C. PCR products were analyzed using 1% agarose gel.

Oral swabs from the IUD users and nonusers as well as vaginocervical swabs from the nonusers (control) were subjected to the same procedures mentioned above.

The data observed were tabulated and statistically analyzed. Chi-square was the test chosen for analysis at .05 level of significance [16].

## 3. Results

*E. gingivalis* was identified in 11 (12.64%) of 87 specimens obtained from the wash of the removed IUDs in IUD user cases, as confirmed by morphology and PCR amplification. The mean duration of IUD use of infected women was 44.55±8.8 months, while the mean duration of IUD use of noninfected women was 42.96±6.95 months. There were no statistical differences between these durations of use ( $p>.05$ ).

*E. gingivalis* was detected in six cervical–vaginal swabs specimens (6.9%) from the control group. On comparing the infected cases in both groups, this difference is not significant ( $p>.3$ ) (Table 1).

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