Original Article

The Role of Mother's Oral and Vaginal Yeasts in Transmission of Helicobacter Pylori to Neonates

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Abstract

Background: Oral cavity has been proposed as an important reservoir of *H.pylori*, being implicated in bacterial transmission through oral-oral route. However, some investigators believe that the newborn acquires *H.pylori* from mother through vaginal delivery. In this study, oral and vaginal yeasts were examined for the intracellular occurrence of *H.pylori* and their possible role in bacterial transmission.

Methods: Sixty nine oral and vaginal yeasts from expecting mothers (39 oral and 30 vaginal) and seven oral yeasts from neonates(6/46 vaginal delivery, 1/43 cesarean) were identified and studied by light and fluorescent microscopy for observing the intracellular bacterium-like bodies(BLBs). Whole DNAs of yeasts were recruited for detection of *H.pylori*-specific genes. Urea breath test (UBT) was performed for detection of *H.pylori* infection in mothers. Stool antigen test (SAT) was used for detection of *H.pylori* antigens in infants' stool at birth and six months of age.

Results: Oral yeasts were isolated more frequently from normally-delivered neonates. The frequency of *H.pylori* genes in mothers' vaginal yeasts was significantly higher than in mothers' oral yeasts. A significant correlation was found between the occurrence of *H.pylori* genes in vaginal yeasts and that in neonates' oral yeasts, occurrence of *H.pylori* genes in mothers' vaginal yeasts or neonates' oral yeasts, and UBT+ results in mothers.

Conclusion: *C.albicans* which colonizes the oral cavity of neonates through vaginal delivery or contact with environment or healthcare workers could be an important reservoir of *H.pylori*. Vaginal yeasts are more potent in accommodating *H.pylori* than oral yeasts. Accordingly, vaginal yeast is proposed as the primary reservoir of *H.pylori* which facilitates *H.pylori* transmission to neonates.

Keywords: *H.pylori* genes, oral yeasts, transmission, vaginal yeasts

Cite this article as: Siavoshi F, Taghikhani A, Malekzadeh R, Sarrafnejad A, Kashanian M, Jamal AS, et al. The role of mother's oral and vaginal yeasts in transmission of *Helicobacter pylori* to neonate. *Arch Iran Med*. 2013; **16(5):** 288 – 294.

Introduction

Studies until now have described that infection of *Helicobacter pylori* is primarily acquired in early childhood.[1–4] Person-to-person spread within the family appears to be the predominant mode of *H.pylori* transmission.[5] Although it is not clear how the bacterial infection initiates for the first time, a considerable number of studies demonstrate that the infected mother is the most important risk factor for childhood infection.[5–7] It is believed that mothers could transmit *H.pylori* to their infants through mouth secretions,[8] using common spoons, the licking of pacifiers, the teats of feeding bottles, or even by chewing or tasting of the child's food.[9] Furthermore, poor hygienic practice during childhood such as lack of tooth brushing habit has been implicated in increasing the risk of *H.pylori* infection.[10] These indicate that oral cavity could be considered as the primary site of colonization and an important reservoir of *H.pylori*. However, there is no strong evidence to support the idea that plaque and saliva create sufficiently favorable conditions for *H.pylori* survival and growth.[11] This is because occurrence of *H.pylori* in the oral cavity has been mainly demonstrated by detection of *H.pylori* genes[12] and not by culture.[13,14] In our previous studies, we could amplify *H.pylori*-specific genes from the total DNAs of gastric[15] and oral[16] yeasts and demonstrated the intracellular existence of *H.pylori* inside the vacuole of *Candida* yeast. It was proposed that *Candida* which thrives in the mucosal surfaces of human body including oral cavity, gastrointestinal(GI), urinary, and genital tract[17] could serve as the reservoir of *H.pylori* and play an important role in bacterial transmission to a new host.[15,16,18]

Candida albicans is the yeast species which commonly colonizes healthy adults and children as well as hospitalized patients. Adherence to mucosal surfaces enables *C.albicans* to selectively colonize the human host.[19] Mucosal surfaces of oral cavity and vulvo-vaginal areas are the first and second most frequently colonized areas of the human body, respectively.[20] The oral carriage rate in healthy individuals has been reported up to 75%.[21] A considerable number (5% – 35%) of healthy women who are completely asymptomatic have positive vaginal culture for *C.albicans* [22] and the level of colonization can reach the most intensive level of colonization than any other body site.[19] Furthermore, the incidence of vaginal yeast colonization during pregnancy has been reported as 25% – 46%.[23,24] *Candida* spp in infants may be acquired vertically from the mother when passing through the birth canal via cutaneous contact or swallowing of fungi.[24,25] It is generally recognized that colonization of newborns with *Candida* occurs at the first few hours of life and the oral colonization is the common-

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Accepted for publication: 28 December 2012

est among babies (26.7%).[26,27]

In this study the role of mothers' oral and vaginal yeasts in transmission of *H. pylori* to neonates was assessed. *Candida* yeasts from oral cavity and vagina of expecting mothers and those from oral cavity of their neonates were examined for the intracellular occurrence of *H.pylori*, using polymerase chain reaction (PCR) for detection of *H.pylori*- specific genes in the total DNA of yeasts. The relationship between harboring *H.pylori*- specific genes by yeasts and the results of mothers' urea breath test (UBT) and neonates' stool antigen test (SAT) were assessed.

Materials and Methods

Patients

108 pregnant mothers referred to delivery wards of Shariati and Shahid Akbarabadi hospitals, Tehran University of Medical Sciences (TUMS), were recruited in the study. Mothers were 21 – 39 years old and asked about dyspepsia complaint. All the patients signed the consent form and the study was confirmed by the Research Ethics Committee of TUMS. Patients included 56 mothers with normal delivery and 52 with cesarean. UBT and sampling for isolation of oral and vaginal yeasts were performed prior to delivery.

UBT

UBT was performed for all the 108 mothers to assess the status of *H.pylori* infection. Instructions were followed according to manufacturer's protocol (CAMPRO Scientific, Germany). Briefly, mothers had to have an empty stomach and no antibiotic consumption for two weeks. Mothers in the cesarean group were tested for UBT two weeks after the end of antibiotic course. Mothers were first asked to blow into the pocket No.1 as a control. Next, they took a 100-mL solution of ^{13}C-urea and blew into the second pocket after 30 minutes. The amount of exhaled $^{13}CO_2$ was measured by the spectrometer (Wagner, Germany). Counts were compared with the controls and those above 5% were considered (+), below 4% (-), and 4% – 5% questionable (Q).

Yeasts isolation and identification

Swabs were taken from the mucosal surfaces of the oral cavity and vagina of 108 mothers and oral cavity of 89 neonates. Oral swabs were rubbed against the tongue, gingival, and oral mucosa. Vaginal swabs were rubbed against 5 – 7 cm of vaginal canal. Swabs in transport medium were transferred to the microbiology laboratory. Oral and vaginal swabs were cultured on Yeast Glucose Chloramphenicol (YGC) agar (Merck, Germany) and incubated at 37 °C for 24 – 48 h. Smears from yeast colonies were stained by Gram's method for observing the typical oval yeasts morphology and possible bacterial contamination. Single colonies of yeasts were subcultured on YGC more than 10 times to remove any bacterial contamination. Pure cultures of yeasts were identified as *Candida* spp, using Chromagar (CHROM agar, France). *C.albicans* produced green and *Candida* spp white-pink colonies. Majority (39/46, 85%) of oral yeasts from mothers in normal and cesarean groups as well as those from neonates were *C.albicans* and the rest (7/46, 15%) *Candida* spp. Vaginal yeasts from mothers in normal and cesarean groups also included 24/30 (80%) *C.albicans* and 6/30 (20%) *Candida* spp.

Light and fluorescent microscopy

Wet mounts were prepared from yeast isolates and examined by light microscopy for observing the persistent occurrence of bacterium-like bodies (BLBs) inside the mother and daughter yeast cells after several subcultures. Photographs were taken at four time intervals from oral yeast O_{27}. Live/Dead BacLight Bacterial Viability Kit (Invitrogen,USA) was used for the preparation of yeast samples for fluorescent microscopy. The O_{27} yeast was grown in 100 mL of culture medium containing yeast extract (5 g) and glucose (20 g) and incubated at 37°C for 48 – 72 h. A 0.5-mL volume of yeast suspension with the turbidity of 0.5 McFarland's standard was mixed with 1.5 µL of fluorescent stain containing equal volumes of SYTO 9 and propidium iodide. After a quick vortex and incubation at dark for 15 min, 10 µL was placed on a glass slide and examined by fluorescent microscope. Photographs were taken at two or four time intervals from live and moving BLBs which appeared green inside the dark vacuole of yeast O_{27}.

Recovery of the intracellular H.pylori from yeasts

In order to cultivate the intracellular BLBs from yeasts and identify them as *H.pylori*, 0.5 mL of acid-washed glass beads were added to 2 mL of 24-h cultures of selected yeasts (O_{27}, O_{22}, V_{27}, V_{22}, $O_{n\text{-}27}$, $O_{n\text{-}22}$) in Brain Heart Infusion (BHI) broth and vortexed. Crude extracts of the disrupted yeasts were cultured on Brucella blood agar with antifungal amphotericin B (4 mg/L) and incubated under microaerophilic conditions at 37°C and observed for bacterial growth for more than three weeks. Because attempts to culture BLBs from disrupted yeasts were not successful, PCR was used for detection of *H.pylori*-specific *16S rRNA* and *vac A s* genes in the whole DNA of yeasts.

Amplification of H.pylori-specific genes from yeasts

DNA extraction from yeasts and a previously PCR-confirmed *H.pylori* (control) was performed according to our previous study[18] and Sambrook's method.[28] Seventy six (46 oral and 30 vaginal) yeasts were examined for the presence of *H.pylori 16S rRNA* and *vac A s1/s2* genes. PCR amplification was carried out in a total volume of 25 µL of 10x PCR buffer (Cinagen,Iran), 2.5 mM $MgCl_2$ (Cinagen,Iran), 200 µM of each dNTP (Cinagen,Iran), 2 U of Taq DNA polymerase (Cinagen,Iran), 0.5 µM of each primer, and 100 ng of bacterial or 500 ng of yeast DNAs. The recruited primers were: HPF > 5'-GCAATCAGCGTCAGTAATGTTC-3' , HPR > 5'-GCTAAGAGATCAGCCTATGTCC-3' for *16S rRNA*,[29] and VA1F > 5'-ATGGAAATACAACAAACACACC-3' , VA1R > 5'-CTGCTTGAATGCGCCAAAC-3' for *vac A(s1/s2)*.[30] Amplification of the *H.pylori* -specific genes from yeasts and control *H.pylori* was performed in 35 cycles as follows: 5 min at 94°C, 1 min at annealing temperatures of 56°C for *16S rDNA* and 54°C for *vac A s* alleles, followed by 1 min at 72°C, and a final extension at 72°C for 10 min. PCR products were electrophoresed and their size was determined, using molecular ladder. The PCR products of *16S rRNA* gene, which were amplified from the oral O_{27} and vaginal V_{27} yeasts of mother and the oral yeast of her newborn $O_{n\text{-}27}$ were sequenced and matched with *H.pylori* published sequences in gene bank by BLAST program (http//:www.ncbi.nlm.nih.gov).

Detection of H.pylori in stool of infants by SAT

SAT kit (Generic assay-Germany) was used for detection of *H.pylori* antigen in neonate's stool samples obtained from 72/108 (66.7%) newborns at birth and 31/72(43%) at six months of age. Samples in 1x PBS were kept frozen (-20°C) until tested. A 100-

mg stool sample was suspended in 0.5 mL of sample diluents, mixed well and 100 µL was added in duplicates to 96-well plates. the First antibody was added and nonspecific antigens were removed by washing. The second antibody labeled with horse reddish peroxidise was added and after washing, enzyme substrate was added and the intensity of colored product was measured at 450 – 620 nm by ELISA reader (Mindray MR.96A- Germany).

Statistical analyses

Chi-square test and One Sample t-test, SPSS version 20 were used to examine the relationship between occurrence of *H.pylori*-specific genes in oral and vaginal yeasts of mothers and oral yeasts of their newborns and the results of UBT and SAT. The correlation between UBT results and dyspepsia was also examined. *P- Values* < 0.05 were considered significant.

Results

Patients' UBT and dyspepsia

In normal delivery group (56 mothers), 30 (53.6%) were UBT-positive, 23 (41.1%) UBT-negative ,and three (5.3%) UBT-questionable (Q). In the cesarean group (52 mothers), 27 (51.9%) were UBT+, 21 (40.4%) UBT-, and four (7.7%) UBT-Q. In the normal delivery group, UBT + mothers included 21 (70%) and nine (30%) with or without dyspepsia, respectively. In cesarean group, UBT + mothers included 16 (59.3%) and 11 (40.7%) with or without dyspepsia, respectively (Table 1). A significant correlation was found between UBT + and dyspepsia in a total of 57/108 (52.8%) expecting mothers ($P = 0.039$).

Yeast isolation

Oral yeasts were isolated from 39/108 (36.1%) mothers; 17/56 (30.35%) from normal delivery group and 22/52 (42.3%) from cesarean group. Vaginal yeasts were isolated from 30/108 (27.8%); 18/56 (32.4%) from the normal delivery group and 12/52 (25%) from those in cesarean group (Table 1). No significant difference was found between the frequency of yeasts in the oral cavity or vagina of the two groups ($P = 0.11$). Seven neonates with oral yeasts included 6/46 (13%) of normal delivery group and 1/43 (2.3%) of the cesarean group. Statistical analysis showed that oral yeasts were isolated more frequently from normally-delivered neonates compared to the cesarean group ($P = 0.0063$).

Light and fluorescent microscopy

Light microscopic observations showed the presence of fast-moving BLBs inside the vacuole of all yeast isolates. Observations on subcultures of O_{27} yeast showed BLBs inside the vacuole of mother as well as daughter yeast cells (Figure 1). Fluorescent microscopy showed live and green BLBs inside the yeast vacuoles. Some yeast vacuoles contained more than one BLBs. BLBs were also present in both mother and daughter yeast cells. Other green parts outside the vacuole could represent the nuclear material or mitochondrial DNA (Figure 2).

PCR

The size of PCR products of *16S rRNA* (519bp) and *vac A s1* (259bp) genes was similar to those of control *H.pylori* (Figure 3). Analysis of the sequenced products revealed 99% – 100% homology between *16S rRNA* gene amplified from yeasts and those from *H.pylori*. *H.pylori*-specific genes, *16S rRNA* and *vac A s1*, were detected in 13/39 (33/3%) oral yeasts, 18/30 (60%) vaginal yeasts, and 5/7 (71%) neonates' oral yeasts. The frequency of vaginal yeasts that contained *H.pylori*-specific genes was almost twice (1.8-fold) that of oral yeasts (Tables 1 and 2). In a group of seven mothers with oral and vaginal yeasts and their seven neonates, 4/7 (57%) of the mothers' oral yeasts, 6/7 (86%) of their

Table 1. Frequency of *H.pylori-16S rRNA* and *vac A s1* genes in mothers' oral (39) and vaginal (30) yeasts and their relationship with type of delivery, UBT, and dyspepsia.

	Isolated yeasts		Occurrence of *H.pylori* genes in yeasts		Total number of mothers with		UBT- positive mothers	
Delivery	**Oral**	**Vaginal**	**Oral**	**Vaginal**	**Dyspepsia**	**UBT +**	**With dyspepsia**	**Without dyspepsia**
Normal	17/56(30.4%)	18/56(32.1%)	6/17 (35.3%)	12/18 (66.7%)	33/56(58.9%)	30/56(53.6%)	21/30(70%)	9/30(30%)
Cesarean	22/52(42.3%)	12/52(23%)	7/22 (31.8%)	6/12 (50%)	27/52(51.9%)	27/52(51.9%)	16/27(59.3%)	11/27(40.7%)
Total	**39/108(36.1%)**	**30/108(27.8%)**	**13/39 (33.3%)**	**18/30 (60%)**	**60/108(55.6%)**	**57/108(52.8%)**	**37/57(64.9%)**	**20/57(35%)**

Table 2. Frequency of *H.pylori-16S rRNA* and *vac A s1* genes in seven oral (O) and six vaginal (V) yeasts of mothers, seven oral yeasts of their neonates (O_n), and their relationship with type of delivery, UBT, and SAT (zero and six months).

Item	Type of delivery	UBT	SAT (0m)	SAT (6m)	Oral yeast	*16S rRNA*	*vac A s1*	Vaginal yeast	*16S rRNA*	*vac A s1*
1	**Normal**	-	-	-	O_6	-	-	V_6	-	-
			-----	-----	O_{n6}	-	-	-----	-----	-----
2	**Normal**	+	-	NS	O_{n-19}	+	+	V_{19}	+	+
			-----	-----	-----	-----	-----	-----	-----	-----
3	**Normal**	+	-	-	O_{22}	+	+	V_{22}	+	+
			-----	-----	O_{n-22}	+	+	-----	-----	-----
4	**Normal**	+	-	+	O_{27}	+	+	V_{27}	+	+
			-----	-----	O_{n-27}	+	+	-----	-----	-----
5	**Normal**	-	-	-	O_{35}	+	+	V_{35}	+	+
			-----	-----	O_{n-35}	+	+	-----	-----	-----
6	**Normal**	-	-	-	O_{n-41}	-	-	V_{41}	+	+
7	**Normal**	+	-	+	O_{46}	-	-	V_{46}	+	+
8	**Normal**	+	-	+	O_{49}	-	-	NY	NA	NA
9	**Cesarean**	-	-	NS	O_{108}	+	+	NY	NA	NA
			-----	-----	O_{n-108}	+	+	-----	-----	-----

m: Month; NS: No sample; NY: No yeast was isolated; NA: Not applicable.

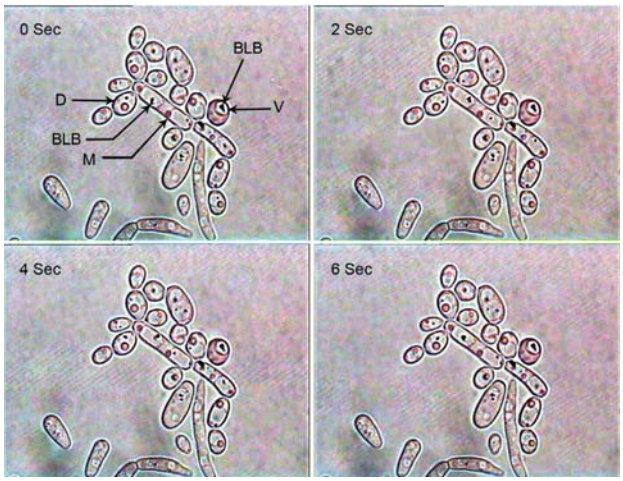

Figure 1. Light microscopy of the oral *C.albicans* yeast (O27). Photographs taken at four time intervals to show fast moving BLBs inside the yeasts vacuoles. BLBs occur inside the vacuoles (V) of mother (M) and daughter (D) yeast cells. Original magnification x1250.

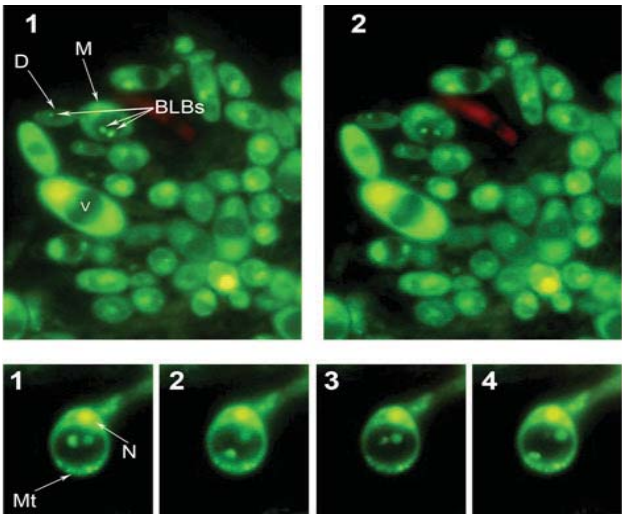

Figure 2. Fluorescent microscopy of the oral *C.albicans* (O27). The live and green BLBs are demonstrated in the yeasts vacuoles (V). Top: Photographs taken at two time intervals show two moving BLBs occurring in a mother cell (M) and one in a daughter cell (D). Bottom: Photographs taken at four time intervals show moving BLBs inside the yeast's vacuole; N: Yeast's nucleus, Mt: Mitochondria. Original magnification x 1250.

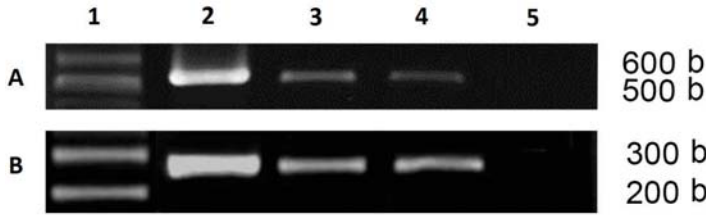

Figure 3. Detection of *H.pylori-16S rRNA* and *vac A s1* genes in O_{27} and V_{27} yeasts. The PCR products of (A) *16S rRNA gene* (519bp) and (B) *vac A s1 gene* (259bp) were amplified. Lanes 1: Molecular ladder, Lanes 2: Control *H.pylori* , Lanes 3: O_{27}, Lanes 4: V_{27} , Lanes 5 : No template.

vaginal yeasts, and 5/7 (71%) of their neonates oral yeasts carried *H.pylori*-specific *16S rRNA* and *vac A s1* genes (Table 2).

H.pylori genes in yeasts and UBT

The frequency of *H.pylori*-specific genes in mothers' vaginal yeasts was significantly higher than in mothers' oral yeasts (P = 0.021). A significant correlation was found between the occurrence of *H.pylori*-specific genes in vaginal yeasts and that in neonates' oral yeasts (P = 0.001). Statistical analysis did not show a significant relationship between the frequency of *H.pylori*-specific genes in mothers' oral yeasts and UBT+ results. However, a significant relationship was found between the occurrence of *H.pylori*-specific genes in mothers' vaginal yeasts and UBT+ results (P = 0.031). Similar significant relationship was found between the occurrence of *H.pylori*-specific genes in neonates' oral yeasts and mothers UBT+ results (P = 0.004).

SAT and UBT

The results of SAT were negative for all 72 stool samples taken at birth and positive for only 3/31 (9.68%) samples taken at six months of age. Out of three mothers with oral and vaginal yeasts as well as their neonates' oral yeasts carrying *H.pylori* genes, two were UBT+ and one neonate was SAT+ at six months of age. No relationship was found between UBT results in mothers and SAT in neonates at birth. However, neonates of 3/5 (60%) UBT+ mothers, two with dyspepsia, became SAT+ at six months of age (Table 2).

Discussion

Several reports describe the occurrence of nonculturable *H.pylori* in the oral cavity and oral-oral route as a possible way of bacterial transmission.[8,9,11–14] However, some investigators consider vertical transmission of *H.pylori* from mother to neonate through vaginal delivery as one important way for the spread of *H.pylori*. Although isolation of *H.pylori* from vaginal mucosa has not been reported until now,[31] it has been proposed that *H.pylori* is able to survive on vaginal mucosa if it could inhabit the squamous epithelium of the oral cavity.[32] Reports indicate that vagina supports the growth of a number of microaerophilic organisms, suggesting the occurrence of a coexisting infection in vagina that favors the growth conditions for *H.pylori*.[33–35] Intracellular symbiotic relationship of *H.pylori* with *Candida* yeast has been proposed as an important strategy for the survival of *H.pylori* outside its well-known niche, the human stomach.[36] *C.albicans* is a ubiquitous organism and normal colonizer of GI and vaginal tract of humans.[37]

The early *Candida* spp colonization of neonates is believed to be mostly due to vertical transmission from mother's vagina to child[24,25,38] and oral colonization is the commonest among babies (26.7%).[39] Newborns also acquire yeasts horizontally within the first few days of life after close contact with mother (e.g., during feeding)[40] or from the environment, primarily the hands of healthcare workers in the hospital.[41]

Results of this study indicate that although vaginal yeasts were isolated with the same frequency from mothers in normal delivery and cesarean groups, only a considerable number of vaginally-delivered neonates carried oral yeasts ($P = 0.0063$). A study on 100 pregnant women, 72 with vaginal delivery and 28 who underwent cesarean, reported that *Candida* spp were isolated from the vaginal mucosa of 47.2% of mothers with normal delivery and the oral cavity of 25% of their newborns. However, among the mothers who underwent cesarean, 46.4% carried *Candida* yeast in their vagina and only 3.6% of their newborns carried yeast in their oral cavity. There was a genotype concordance between the yeasts from mothers vagina and those from the oral cavity of newborns in 23.5% of normal delivery cases but none in the cesarean cases.[42] The number of vaginal yeasts carrying *H.pylori* genes was almost twice (1.8 fold) that of oral yeasts ($P = 0.036$). Statistical analysis showed a significant correlation between the frequency of *H.pylori* genes in vaginal yeasts and that in oral yeasts of normally delivered neonates ($P = 0.006$). This correlation was not found between mothers' oral yeasts and neonates' oral yeasts. These results indicate that yeasts from mother's vagina and those isolated from neonate's oral cavity might have a common source. Furthermore, higher frequency of vaginal yeasts with *H.pylori*-genes, compared with oral yeasts might indicate that yeast colonizers of vaginal mucosa of mothers could serve as primary reservoirs of *H.pylori* which facilitate bacterial transmission from mother to child.

Among 108 expecting mothers, 57 (52.8%) had complaint of dyspepsia and were UBT+. There was a significant correlation between UBT+ and dyspepsia in mothers ($P = 0.039$). No significant relationship was found between UBT+ and SAT+ or dyspepsia and SAT+ results, indicating that further study with higher number of samples is warranted. Neonates from 3/5 (60%) UBT+ mothers (2/3 with dyspepsia) became SAT+ at six months of age. Of these three UBT+ mother-SAT+ neonate pairs, two mothers' vaginal yeasts, one mother's oral yeast, and one neonate's oral yeast carried *H.pylori* genes. Statistical analyses showed a significant correlation between UBT+ results in mothers and occurrence of *H.pylori* genes in mothers' vaginal yeasts or that in neonates' oral yeasts. In a study from Japan, 51 children born from *H.pylori*-positive mothers were followed up for five years. Four out of 51 became positive by SAT in the first two years and 1/51 at four years and four months of age. Fingerprinting revealed that *H.pylori* isolates from children were similar to those of their corresponding mothers.[43] Two reports from Germany showed a strong association between the maternal infection status and the risk of *H.pylori* transmission to infant, emphasizing the key role of mothers in spread of *H.pylori* within the family.[44,45] Accordingly, it is proposed that neonates born from UBT+ mothers colonized with vaginal *C.albicans* harbouring *H.pylori* could be at higher risk of *H.pylori* infection.

Light microscopy showed the occurrence of fast moving BLBs inside the vacuole of yeasts. Fluorescent microscopy confirmed that BLBs were alive. Although BLBs were not culturable, detection of *H.pylori*-specific genes in total DNA of yeasts confirmed their identity as *H.pylori*. Thirteen out of 39 (33.3%) of mothers oral and 18/30 (60%) of their vaginal yeasts and 5/7 (71%) of neonates' oral yeasts contained *H.pylori*-specific genes; *16S rRNA* and *vac As1*. These results demonstrate the intracellular occurrence of *H.pylori* inside the yeast *Candida*. Intracellular association of *H.pylori* with *Candida* yeast described in this study is one example of the complex interaction between bacteria and fungi. Further examples include intracellular existence of *Paenibacillus* spp in *Laccaria bicolor* S238N,[46] *Cyanobacteria* in *Geosiphon pyriforme*,[47] *Burkholderia* in *Gigaspora* spp,[48] and *Scutellospora* spp.[49] Unravelling the details of interaction between bacteria and fungi has been hampered mainly due to nonculturability of their endosymbiotic bacteria. The true bacterial nature of these bacterium-like organisms has only been confirmed recently with the help of PCR amplification of prokaryotic genes from total DNA of fungi and sequencing.[48,50] Light and fluorescent microscopy observations on consecutive subcultures of yeasts showed the occurrence of *H.pylori* cells inside the vacuoles of mother as well as daughter yeast cells. Some yeasts had more than one *H.pylori* cells inside their vacuoles. These results indicate the potential of *H.pylori* cells to multiply and transmit to the next generations of yeasts. Confocal microscopy showed that endosymbionts of *Gigaspora* and *Scutellospora* spp were transmitted vertically in their fungal host from generation to generation.[51] Although, vertical transmission is a very common phenomenon for endobacteria associated with animals,[52] reports indicate that acquisition of endobacteria by AM fungi during evolution has occurred as a rare event. However, the association between endobacterium and its fungal host is so intimate that the bacterium is not capable of independent survival outside the host and thus needs to be vertically transmitted to the subsequent generations of its host.[53] In this study, microscopic observations on new generations of yeasts and amplification of *H.pylori* genes from consecutive generations of yeasts indicated that new yeast cells might inherit the intracellular *H.pylori* as part of their vacuolar contents. It has been demonstrated that inheritance of vacuole in yeast is a highly regulated process which ensures its inheritance by the daughter cells.[54] Accordingly, it is proposed that inheritance of yeasts carrying *H.pylori* in their vacuole, from mother to neonate could serve as a safe mechanism to ensure transmission and spread of *H.pylori*.

Interaction of bacteria and yeast has been mostly described as surface adherence in the formation of infectious biofilms which are resistant to host immune system and antibiotics. Examples include multispecies infection of an artificial urethral sphincter by *C.albicans*, *Streptococci* and *P.aeruginosa*,[55] biofilms found on the gastric portion of nasogastric tubes with Gram-positive cocci and Gram-negative rods and yeast,[56] concurrence of *Candida* and *Lactobacilli* in the oral biofilms of immunosuppressed patients,[57] and adhesion of *C.albicans* and *S.sanguis* and *S.salivaris* to acrylic surfaces of dentures.[58] It appears that actively respiring *C.albicans* in the oral cavity reduces the oxygen tension level and provides stimulatory factor for *Streptococci* while bacteria provide nutrients that promote fungal growth.[59] These reports indicate that *C.albicans* with extensive distribution on human skin and mucosal surfaces is frequently implicated in mixed bacterial-fungal infections. Future studies on interactions between these two microorganisms will reveal their crucial role in humans' health.[60,61]

Results of this study showed that the frequency of vaginal yeasts harboring *H.pylori* genes was higher than oral yeasts. This might

reflect that the vaginal environment is more favorable for yeasts to retain their intracellular *H.pylori*. It appears that provision of appropriate pH, glucose, and estrogen by vaginal environment promotes the growth of *C.albicans*.[62] Estrogen which increases during pregnancy reduces the secretion of IgG and IgA antibodies into vaginal fluids[63] and peripheral blood lymphocyte responses.[64] Estrogen also increases the vaginal avidity for *C.albicans* because yeast cells possess cytoplasmic receptors for female reproductive hormones.[65] Accordingly, results of this study propose that vaginal yeast is a potent reservoir of *H.pylori* and could play a crucial role in transmission of *H.pylori* from mother to neonate. Furthermore, neonates born from UBT+ mothers colonized with *C.albicans* in vagina could be at higher risk of *H.pylori* infection. Infection of infants by *C.albicans* through vertical transmission is an old discussion,[66] however, recent reports indicate that heavily colonized intrauterine devices (IUD)[24,67,68] and preferential adherence of *Candida* yeast to intermediate layers of vaginal tract that are increased during pregnancy, could increase maternal fungal colonization and the chance of exposure of vaginally-delivered infants.[69] Accordingly, to prevent vertical transmission of yeast / *H.pylori*, treatment of maternal IUD infections and *Candida* vaginosis during pregnancy might decrease the inocula to which the infant is exposed.[66] Furthermore, careful attention to hand hygiene of healthcare workers may play a major role in reducing the fungal transmission.[41,70] Oral hygiene of mother and child could also help to reduce yeast content of the mouth and control of *H.pylori*.

Acknowledgments

This study was funded by Research Council of University of Tehran and Digestive Disease Research Institute,TUMS. Parts of this study have been presented as two posters in XXVth International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer (2012). The authors have no conflict of interest.

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