

## RESEARCH ARTICLE

**Salivary ABO antigens and risk of microbial vaginosis**Azhar Hatif Oleiwi Al-Kuraishi,<sup>1</sup> Salma L. Dahash,<sup>2</sup> Manal Madany A. Qader<sup>3</sup>**Abstract****Objective:** To investigate the effect of blood group ABO antigens on the risk of vaginosis.**Methods:** The cross-sectional study was conducted at the Department of Obstetrics and Gynaecology, Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from April 2016 to June 2017. Two vaginal swabs and 1ml of stimulated saliva from women aged 16-46 years were collected. The first swab was used for direct wet smear examination, while the second swab was cultured on aerobic and facultative anaerobic cultures on appropriate media. SPSS 25 was used for data analysis.**Results:** Of the 269 patients with a mean age of 30.7±6.2 years, 52(19.3%) were positive and 217(80.7%) were negative for ABO antigen. The duration of vaginosis symptoms were observed after 7-13 days in both positive and negative groups (p=0.24). The main symptom in women with positive ABO was vaginal pain, while it was a foul-smelling vaginal discharge and itching in women with the negative status (p=0.0001). Single bacterial species growth was obtained from 32(61.5%) positive patients and 81(37.3%) negative patients.**Conclusion:** ABO secretory status could increase defence against microbial vaginosis.**Keywords:** ABO antigens, Saliva, Microbialvaginosis. (JPMA 69: S-50 (Suppl. 3); 2019)**Introduction**

Vaginal discharge is a symptom caused by either physiological or pathological reasons. The physiological causes are the most common and usually are clear, non-offensive and non-pruritic.<sup>1</sup> Pathological causes include infections, such as bacterial vaginosis, candidiasis, trichomoniasis, and non-infective, like foreign body, cervical ectopic, and malignancy.<sup>2</sup> About half of vaginosis cases are bacterial which is due to excessive growth of mixed anaerobes that replace normal vaginal lactobacilli. Bacterial vaginosis occurs spontaneously in both sexually active and inactive women.<sup>2,3</sup> Acute vulvo-vaginal candidiasis may be a common cause of vaginosis in about 80% of infections, which is caused by overgrowth of *Candida albicans*. It is most common in women aged 20-30 years and in pregnancy as oestrogens promote its growth.<sup>4</sup> Moreover, *Trichomonas (T.) vaginalis* is the least cause of vaginosis which is exclusively sexually transmitted and accounts for 3% of the infected women.

The word 'secretor' is used for an individual who secretes ABO blood group antigens in body fluids such as saliva, sweat, tears, gastric juice, semen, urine, etc., and it is completely independent of the blood type. While the individual who has no ability to secrete those antigens in

body fluids is called a 'non-secretor'.<sup>4,5</sup> The absence of these antigens increase the susceptibility to a number of diseases. The secretion of ABO antigens into saliva and mucus offers a degree of protection against parasitic and bacterial infections. Indeed, different studies have illustrated the influence of ABO antigens on diverse diseases as 68% of those having the disease were non-secretors.<sup>6</sup> A study showed that the absence of ABO antigens in saliva increases the susceptibility to *Neisseria (N.) gonorrhoea* infection,<sup>7</sup> as well. One study demonstrated that the secretory status plays an intrinsic role in the prevention of *Helicobacter (H.) pylori* infection as ABO antigens improve mucosal innate immune system.<sup>8</sup> Besides, one study found that the absence of salivary ABO antigens increases the susceptibility to urinary tract infections (UTIs).<sup>9</sup>

The current study was planned to investigate the effect of blood group ABO antigens on the risk of vaginosis.

**Subjects and Methods**

The cross-sectional study was conducted at the Department of Obstetrics and Gynaecology, Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from April 2016 to June 2017. After approval from the institutional ethics committee, and informed written consent from the subjects, two vaginal swabs and 1ml of stimulated saliva were collected from married women aged 16-46 years who complained of symptoms akin to vaginosis. Data was gathered regarding age, marital status, period of marriage, number of children, education level,

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occupation, period of infection, and main symptoms. The first vaginal swab was used for direct microscopically wet smear examination. It was smeared upon a microscope slide by placing the specimen on a glass slide and mixed with normal saline.<sup>10</sup> The second vaginal swab was cultured on aerobic and facultative aerobic on appropriate media. At the end of the incubation period, all the isolates were diagnosed according to well-known established microbiological methods based on morphological features, gram-staining method and biochemical tests.<sup>11</sup>

The stimulated saliva sample was collected into a sterile glass jar. The saliva from each woman was boiled for 20 minutes, centrifuged at 2000 rpm for 10 minutes and the supernatant was stored briefly at 20°C till it was tested for the presence of ABO antigens by using the haem-agglutination inhibition assay. Briefly, the test used the anti-A, B and D sera. It is based on the principle that if ABO antigens are present in the saliva, they will bind with antibodies that present in the anti-sera added. So, the antibodies are not available in the mixture (saliva and antisera) to agglutinate with red blood cell (RBC) suspension and the subject is a positively ABO antigen secretory and vice versa.<sup>12,13</sup>

Data was analysed using SPSS 25, and was presented as frequencies, percentages, mean, standard deviation (SD) and range. The significance of difference in qualitative data was tested using Pearson Chi-square test with the application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was set at  $p < 0.05$ .

## Results

Of the 269 patients with a mean age of  $30.7 \pm 6.2$  years, 52(19.3%) were positive and 217(80.7%) were negative for

Table-1: Characteristics of the study sample.

Characteristics	No (%)	M $\pm$ SD
Age (years)	<20y	7(2.6)
	20-29	109(40.5)
	30-39	125(46.5)
	= >40y	28(10.4)
	Mean $\pm$ SD	30.7 $\pm$ 6.2
Education	Illiterate	3(1.1)
	Primary	59(21.9)
	Intermediate	115(42.8)
	Secondary	69(25.7)
Occupation	College	23(8.6)
	Employed	30(11.2)
Duration of marriage (years)	Housewife	239(88.8)
		8.2 $\pm$ 5.5 (1-30)
Number of children	No	32(11.9)
	One	41(15.2)
	Two	59(21.9)
	Three	92(34.2)
	Four and more	45(16.7)
Number of abortions	No	197(73.2)
	One	57(21.2)
	Two & more	15(5.6)
Secretary status	Positive	52 (19.3%)
	Negative	217 (80.7%)

SD: Standard deviation.

ABO antigen. The demographic and clinical characteristics of each patient was noted (Table-1).

The duration of vaginosis ranged 3-60 days. The higher proportion was in the range of 7-13 days in both positive and negative ABO secretory status, but the difference with 1-6 days and >14 days range was not significant (Table-2).

Supra-pubic or vaginal pain was the main symptom in

Table-2: The main symptoms present in women included in this study according to their salivary ABO antigens secretory status.

Symptoms		ABO Secretory		P
		Positive No (%)	Negative No (%)	
Suprapubic or vaginal pain	No	15(9.8)	138(90.2)	0.0001*
	Present	37(31.9)	79(68.1)	
Vaginal discharge	No	40(52.6)	36(47.4)	0.0001*
	Present	12(6.2)	181(93.8)	
Foul smell	No	47(31.3)	103(68.7)	0.0001*
	Present	5(4.2)	114(95.8)	
Itching	No	49(21.7)	177(78.3)	0.025*
	Present	3(7.0)	40(93.0)	
Duration of symptoms (days)				0.25
	6-Jan	15(27.3)	40(72.7)	
	13-Jul	35(17.2)	168(82.8)	
>14		2(18.2)	9( 81.8)	

Table-3: The results of direct microscopic examination of women included in this study according to their salivary ABO antigens secretory status.

Direct Exam		ABO Secretary		P
		Positive No (%)	Negative No (%)	
Pus cells	Few	40(60.6)	26(39.4)	0.0001*
	[+]	10(12.7)	69(87.3)	
	[++]	2(2.3)	84(97.7)	
	[+++]	-	38(100)	
Epithelial cells	Few	23(41.1)	33(58.9)	0.00001*
	[+]	20(20.6)	77(79.4)	
	[++]	8(10.5)	68(89.5)	
	[+++]	1(2.5)	39(97.5)	
RBCs	Few	52(22.0)	184(78)	0.011*
	[+]	-	29(100)	
	[++]	-	4(100)	
Trichomonas vaginalis	No	46(19.7)	187(80.3)	0.6
	yes	6(16.7)	30(80.3)	

RBC: Red blood cells.

Table-4: The results of culture of vaginal swabs of women included in this study according to their salivary ABO antigens secretory status.

		ABO Secretary		P
		Positive No (%)	Negative No (%)	
Bacterial culture	Mixed	4(7.7)	130(59.9)	0.0001*
	Single	32(61.5)	81(37.3)	
	No	16(30.8)	6(2.8)	
E. coli	Positive	16(30.8)	113(52.1)	0.006*
	Negative	36(69.2)	104(47.9)	
Staphylococcus aureus	Positive	9(17.3)	63(29.0)	0.086
	Negative	43(82.7)	154(71.0)	
Streptococcus pyogenes	Positive	6(11.5)	52(24.0)	0.050*
	Negative	46(88.5)	165(76.0)	
Klebsiella spp.	Positive	1(1.9)	34(15.7)	0.008*
	Negative	51(98.1)	183(84.3)	
Proteus spp.	Positive	1(1.9)	29(13.4)	0.019*
	Negative	51(98.1)	188(86.6)	
Pseudomonas spp.	Positive	-	28(12.9)	0.006*
	Negative	52(100)	189(87.1)	
Candida albicans	Positive	7(13.5)	51(23.5)	0.114
	Negative	45(86.5)	166(76.5)	

women with positive ABO status, while foul-smelling vaginal discharge and itching were observed in women with negative status ( $P < 0.05$ ) (Table-3).

Direct microscopic examination indicated the presence of RBCs followed by pus and epithelial cells in both positive and negative ABO secretory status ( $p < 0.05$ ). The number of these cells was higher in patients with negative status (Table-4). Also, 36(13.38 %) women were infected with *T. vaginalis*; 6(16.6%) had positive status and 30(83.3%) had negative.

Swab culture illustrated that 16(30.8%) swabs gave no growth from positive ABO status, and 6(2.8%)

from those with negative status. Single bacterial species growth was obtained from 32(61.5%) in positive and 81(37.3%) in negative group, while mixed growth was present in 4(7.7%) from positive and 130(59.9%) from the negative group. *Escherichia (E.) coli* was the predominant bacterial species present in both positive and negative groups, followed by *Staphylococcus (S.) aureus*, *Streptococcus pyogenes*, *Klebsiella (K.) spp*, *Proteus spp*, and *Pseudomonas (P.) spp.*, which were less frequent species. *Candida (C.) albicans* was present in 7(13.5%) from positive and 51(23.5%) from negative group (Table-4).

## Discussion

The current study explored the relationship between vaginosis and ABO antigens, demonstrating the important role of these antigens in resistance against vaginal infections. These were obvious in the severity of symptoms, numbers and types of inflammatory pus and epithelial cells, and/or infectious organisms. Even the duration of the infection was affected by the presence or absence of these antigens. These findings are in agreement with a study that disclosed a significant correlation between the absence of ABO antigens in the saliva and the risk of vaginosis.<sup>14</sup>

Moreover, oxidative stress and bacterial infections are more pronounced in patients who lack ABO antigens in body fluids as oxidative stress affects the salivary content of protective antigens.<sup>15,16</sup>

ABO blood group antigens are glycol-sphingolipids that contain a lipophilic portion, ceramide, which is attached to a variable carbohydrate chain extending out from the cell surface and plasma membrane.<sup>17,18</sup> These are important antigens which are found on the cell surfaces of erythrocytes, epithelial cells of the urinary, vaginal, gastrointestinal, buccal cavity, as well as respiratory tracts.<sup>19</sup> As a result, this will affect the attachment capacity of infectious organisms and may lead to minimising the manifestation associated with these pathogens. Disease risk factors associated with secretor status may result in part because many viruses, bacteria, fungi and protozoa initiate infection by non-covalent binding to certain mucosal cell surface carbohydrate-binding proteins.<sup>20</sup> Many previous studies have demonstrated an increased risk of infections in non-secretors compared to the secretors.<sup>9</sup> There are some materials extracted from uro-epithelial cells of the non-secretors may consider for the increased binding of *E. coli* and lead to their increased susceptibility to recurrent UTI.<sup>21</sup> These findings were similar to our results which showed that the primary infective bacteria were *E. coli* in ABO non-secretors. Several studies have suggested that the increased susceptibility to different diseases in non-secretors may be elucidated by genetic factors influencing the density and/or specificity of bacterial receptors available to mediate colonisation of their cells.<sup>22</sup> This might be attributed to the uro-epithelial cells taken from non-secretors that show enhanced *E. coli* adherence when compared with cells from the secretors.<sup>23</sup> Also, there is evidence suggesting that ABO non-secretors have lower levels of immunoglobulin-G (IgG).<sup>24</sup> Other researchers considered IgA concentrations to be significantly lower in the non-secretors than in the secretors.<sup>25</sup>

## Conclusions

Secretion of ABO antigens in the saliva and other body fluids in women increased defence mechanism against microbial vaginosis. Also, the presence of these antigens minimised the severity of symptoms in women with vaginosis.

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