

Gardnerella vaginalis and breast cancer

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Citation

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Abstract

G. vaginalis was suggested to be an associated factor in carcinogenesis. We have studied 41 breast disease (31 breast cancer and 10 fibrosing adenomatosis) patients for the presence of G. vaginalis by PCR in tumor, blood and saliva. G. vaginalis was detected in 46% of saliva samples, 44% of blood samples, and 56% of tumor; in 85% cases at least one of patient's samples was found to be G. vaginalis positive. For the first time, a relationship between G. vaginalis and breast disease was shown

INTRODUCTION

Bacterial infections traditionally have not been considered major causes of cancer, however, a number of studies [1, 2, 3] show that some bacteria might be associated with cancer. We suggested that bacterial infection could be one of the reasons for breast cancer incidence. Preliminary PCR-analysis of tissue, blood and saliva samples of 10 breast cancer patients for such common pathogens as Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealiticum, Toxoplasma gondii and Gardnerella vaginalis was conducted. This analysis showed, that except for G. vaginalis, the named bacteria either were not detected, or were rare detected in patients' samples. At the same time, G. vaginalis was detected in the samples of all patients, with 9 out of 10 cases being found in the tumor. Therefore, it was hypothesized that G. vaginalis could be one of the factors influencing breast cancer pathogenesis. The present research deals with examination of this supposition.

MATERIALS AND METHODS

SUBJECT SOURCE

Breast disease (BD) group consisted of 41 patients of the Institute of Oncology of Moldova, who were recommended tumor resection, according to the primary diagnosis. There were no further restrictions when organizing this group. After the confirmation of the diagnosis, this group was divided into two subgroups: cancer group – 31 patients and fibrosing adenomatosis (FAM) group – 10 patients. The cancer group itself was divided into four subgroups, according to cancer stage (4 patients with T1 cancer stage, 19 patients with T2 stage, 4 patients with T3 stage, and 4

patients with T4 stage). The cancer stage was determined according to the International Classification of Diseases for Oncology, WHO Geneva (ICD-0-C50).

All patients were informed about the conducted research, and gave a written consent for it, in accordance with the legislation of the Republic of Moldova (The Law of Patient's Rights in Biomedical Research).

Control group consisted of 30 healthy individuals (13 individuals from Institute of Genetics and Plant Physiology and 17 from Institute of Oncology). Healthy status for the control group was determined by questionnaire.

Uro group was formed based on retrospective studies of 40 patients (21 women, 19 men), who sought medical help for urogenital diseases [4]. The probabilities of G. vaginalis detection in these samples are equal for males and females, so the calculations were done for the whole group.

SPECIMEN COLLECTION

Blood and saliva collection from BD group patients was performed in the Institute of Oncology of Moldova 1 – 2 hours before tumor resection, and tissue samples – after tumor resection. In case of the control group, only saliva was collected for the study. All samples were stored and transported at +4°C. Time between sample collection and DNA isolation did not exceed 24 hours.

DNA ISOLATION AND PCR-ANALYSIS.

DNA from blood and saliva was isolated using lysis buffer containing 5M guanidinium thiocyanat, 50mM Tris (pH 7.5), 10mM EDTA, 0.5% Triton X-100, then extracted using

equal volume of phenol:chlorophorm (vol/vol) and then chlorophorm. DNA was precipitated by 2.5 volumes of ice-cold 96% ethanol in the presence of 0.3M ammonium acetate. Tumor was grinded in liquid nitrogen with addition of lysis buffer, and then extracted as above.

PCR-analysis of Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealiticum, Toxoplasma gondii and Gardnerella vaginalis in the course of preliminary testing was conducted using corresponding tests systems from InterLabServis (Moscow, Russia).

To improve the sensitivity of G. vaginalis detection, the analysis was done by nested-PCR. To identify G. vaginalis, we used primers complementary to the sequence encoding a heat shock protein 60 (hsp60) gene. Primers were made by Alpha DNA (Montreal, Quebec, Canada). Primers used for the first round: forward primer 5'-ATCCTGAAGTTGGCGAAAAGAT-3', reverse primer 5'-AACCTTGCCAGAAGTGAGCA-3'; second round: forward primer 5'-CTTTGGATAAGGTTGGTCAGGAC-3', reverse primer 5'-AATGTATGGGTCTTCAAGAACAGC-3' (amplified fragment length is 167 bp). Both rounds were 30 cycles, with 60°C annealing temperature. Each of the samples was analyzed twice. PCR products were analyzed by agarose gel electrophoresis.

As a positive control DNA template of G. vaginalis from the kit (B-7-100) InterLabServis (Moscow, Russia) was used. As a molecular size marker, Fermentas (Letonia) 100bp ladder was used. PCR was carried out in the programmed thermostat (Pushchino, Russia)

STATISTICAL ANALYSIS

Odds ratio (OR) and relative risk (RR) with 95 % CI (Confidence Interval) was carried out using medcalc software (http://www.medcalc.be/calc/odds_ratio.php). We calculated the Yates-corrected chi-square (χ^2) and 95% CI, according to Glantz [5], P values for χ^2 were obtained using the χ^2 test (Excel). Exact binomial confidence intervals for the data not satisfying $np > 5$, $n*(1-p) > 5$ (p = probability of success in n trials), were calculated by the graph of Binomial distribution for Confidence intervals for ratio [5].

RESULTS

Fig.1A shows an example of G. vaginalis detection in tumor tissue, blood and saliva. During sample analysis, a DNA fragment of the same size, as on control DNA template, was synthesized. Fig. 1B shows, that in the given reaction

conditions, G. vaginalis is detected up to the concentration of 1 DNA molecule per the sample (lanes 1-5). Besides, lack of the signals in the samples 6-8 indicates lack of contamination during reaction.

Figure 1

Figure 1: Nested-PCR results. A - DNA from breast cancer patients' samples. Lane M is a 100-bp ladder. Lane 1 - negative control; 2 - positive control (DNA); 3, 4, 5 - blood, saliva, tumor of one patient; 6, 7, 8 - blood, saliva, tumor of another patient. B - sequential 2x dilution of DNA (positive control). Template DNAs in lanes 1 to 5 were in amounts of 16, 8, 4, 2, 1 molecules respectively. Lanes 6-8 DNA is not detected.

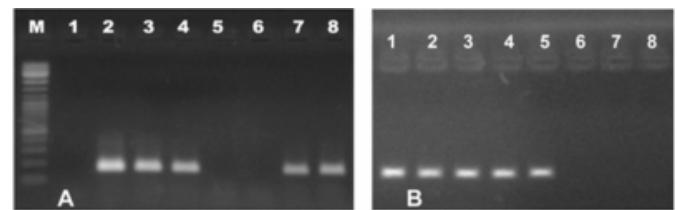


Table1 shows the results of G. vaginalis PCR analysis of different patient groups in different samples (blood, saliva and tumor). Positive cases were counted for each of the analyzed samples, as well as for the combinations: Blood and saliva (variants where G. vaginalis was detected in both or either sample were considered positive) and Total positive cases (G. vaginalis was detected in any of the samples - blood, saliva, tumor). The same approach was used to analyze the group with fibrosing adenomatosis.

Figure 2

Table1: in patients with fibrosing adenomatosis and breast cancer at different stages of carcinogenesis

Group	Number of cases	Positive cases								†Total positive cases	
		Tumor		Blood		Saliva		*Blood and saliva		n	%
		n	%	n	%	n	%	n	%		
Total (Breast diseases)	41	23	56	18	43	19	46	31	76	35	85
Breast cancer	31	17	55	15	48	10	32	22	71	26	84
Stage T 1	4	4	100	2	50	1	25	3	75	4	100
Stage T 2	19	10	53	10	53	6	32	14	74	17	89
Stage T 3	4	2	50	2	50	1	25	2	50	3	75
Stage T 4	4	1	25	1	25	2	50	2	50	2	50
Fibrosing adenomatosis	10	6	60	3	30	9	90	9	90	9	90
Control group	30			2‡	7	2	7	3,3‡	11		
Uro group	40			12	30	12	30	20	50		

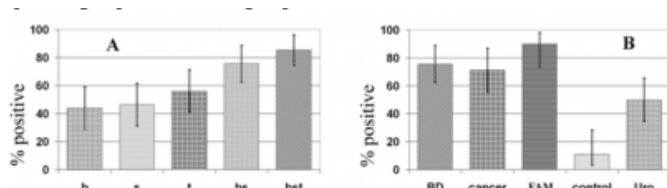
* Number of positive cases when both blood and saliva were considered. Variants where G. vaginalis was detected in both or either sample were considered positive.
 † G. vaginalis was detected in any of the samples: - blood, saliva, tumor
 ‡ calculated values of G. vaginalis detection for control group

Since G. vaginalis detection in control group was carried out in saliva only, the Uro-group, based on retrospective analysis, for which blood and saliva were analyzed, is

introduced to the table [4]. For the Uro-group, the ratio of *G. vaginalis* detection frequency in blood, saliva or either of these samples is 1:1:1,67. Assuming that detection of *G. vaginalis* in any sample of one patient indicates his infection, the Uro-group analysis shows that testing both saliva and blood increases the reliability of infection detection. That is why, in the present research of breast disease group, we tested blood and saliva, besides tumor. The ratio of *G. vaginalis* detection in breast disease group in blood, saliva, or either of these samples is 1:1:1,63. Similarity of the ratios of detection frequencies of *G. vaginalis* in blood, saliva, or either of these samples for Uro (2000-2002) and BD (2007-2008) groups, indicate temporal stability of the frequencies of detection of *G. vaginalis* for different samples. So, we assume it is possible to introduce the calculated values of *G. vaginalis* in “blood” and “blood and saliva”, using the mean values’ ratio 1:1:1,65, for the control group, to the table. Data presented in Fig. 2A confirm that testing several samples is preferable for *G. vaginalis* detection.

Figure 3

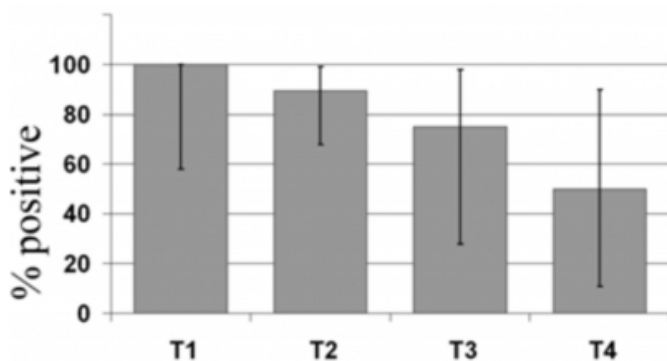
Figure 2: detection in different groups and samples.



A – frequency of bacterial detection in breast disease group for each of the analyzed samples (b, s, t), as well as for the combinations (bs, bst); B - distribution of positive cases (bs) in different patient groups and control group .

Figure 4

Figure 3: Distribution of total positive cases according to breast cancer stages



The frequency of bacterial detection either in blood (b) – 43%, or in saliva (s) – 46% is practically similar, and

increases substantially (1.6 times) when both these data (bs) are considered. The probability of bacterial detection further increases, if *G. vaginalis* in tumor detection is also considered (bst). Fig. 2B shows that the frequency of *G. vaginalis* detection in BD ($\chi^2=26.43, P < 0.001$), cancer ($\chi^2=20.18, P < 0.001$) and FAM ($\chi^2=18.43, P < 0.001$) groups significantly exceeds the frequency of detection of the bacterium in control group (blood and saliva). Fig. 3 shows the results of *G. vaginalis* detection according to breast cancer stages. Frequency of bacterium detection decreases from 100% in T1 to 50% in T4. One of the differences between T1-T4 group patients is that patients of T2, T3, T4 groups received treatment, including polychemotherapy. Average number of polychemotherapy courses for 1 patient for each stage is: T1 - 0, T2 - 0.7, T3 - 3.5 and T4 - 2.5. The distribution of the patients according to the disease stage is similar to the mean distribution T1-8%, T2-49%, T3-28%, T4-16% per year in Moldova (data of the Institute of Oncology of Moldova for 2006).

DISCUSSION

G. vaginalis is a gram-negative to gram-variable bacteria that can be recovered from the normal vaginal flora of one out of every two women. There is now a greater appreciation of *G. vaginalis* as a cause of extrvaginal infections [6] and, to our knowledge, only Mikamo et al. [7] pointed out a relation between *G. vaginalis* and cancer, particularly, cervical cancer.

Data presented in the given research demonstrate a high frequency of *G. vaginalis* detection in patients with breast disease. The high values of odds ratio, OR=21.19 (95 % CI, 5.10-88.06; P < 0.0001) for cancer group and OR=26.87 (95 % CI, 6.68-108.03; P < 0.0001) for BD group compared with the control group (blood and saliva) support a possible association of *G. vaginalis* with breast disease. Even in the analysis of the infection for the BD group in saliva only, compared to the control group, OR=12.09 (95 % CI, 2.54 - 57.56; P < 0.002). The frequency of detection of the bacterium exceeds not only the frequency of detection in control group, but also that in a Uro group of patients with urogenital infection ($\chi^2=4.65, p < 0.02$), with an expectedly high *G. vaginalis* detection. For BD group compared (bs) with Uro group relative risk (RR) was 1.5 (95 % CI, 1.06-2.16; P < 0.025).

Of importance is a rather high frequency of *G. vaginalis* detection in tumor of cancer group patients (p=0.55; 95% CI, 0.37-0.72). Besides, frequency of *G. vaginalis* detection in

tumor FAM group is similar to the frequency of the detection of the bacterium in tumor of cancer group. This provides evidence that *G. vaginalis* may be involved in breast disease pathogenesis.

Application of polychemotherapy for the patients of T2-T4 groups could lead to decrease of concentration or disappearance of *G. vaginalis*, resulting in the decrease of its detection. At the same time, the percentage of *G. vaginalis* detection in patients with diagnosis T1 who did not receive this treatment, is close to 100. The frequency of *G. vaginalis* detection in FAM group, patients of which did not have polychemotherapy, supports this assumption.

In this research, the results of *G. vaginalis* detection in patients with breast cancer and fibrosing adenomatosis are presented for the first time. It is reasonable to assume an association between *G. vaginalis* both the breast cancer and fibrosing adenomatosis, but the role of *G. vaginalis* in these diseases is not clear, and requires further investigations.

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