Novel application for ion mobility spectrometry: diagnosing vaginal infections through measurement of biogenic amines

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Abstract

A method for diagnosis of bacterial vaginosis (BV) and other vaginal infections, based on measurement of biogenic amines present in a sample of vaginal fluid by ion mobility spectrometry (IMS) was developed. Sample introduction is through a two step procedure: addition of alkaline solution to release the volatile amines followed by heating and acid addition for emanation of the semi-volatile amines. Addition of \(n\)-nonylamine vapors to the carrier gas stream helps control the ionization processes and enhances the selective response to amines, even in the complex environment of biological matrices. A software package was developed for acquisition, storage and processing of the mobility spectra and for providing a diagnosis based on a table of rules. We report the results from testing of 210 samples of vaginal discharge fluid that were diagnosed by a gynecologist according to the widely used reference method (Amsel test) and by the new IMS method. The new method is rapid (less than 2 min per sample), has a high sensitivity (few False Negatives) and specificity (few False Positives) with an accuracy of >95% for BV. The use of this method can reduce the incidence of misdiagnosis, particularly when trichomoniasis is confused with bacterial vaginosis.

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1. Introduction

Vaginitis is the general term used to describe vaginal infections that are usually accompanied by a myriad of clinical symptoms, such as pain, itching, irritation, a burning sensation and occasionally vaginal discharge with an unpleasant odor [1–3]. There are several types of vaginal infections, but the three most common forms are bacterial vaginosis (BV), candidiasis (yeast infection) and trichomoni-
of yeast infection), inappropriate personal hygiene habits (frequent douching or lack of cleanliness), personal stress and several other reasons. Treatment of these infections is quite effective, if the proper medication is administered correctly, and the natural balance may usually be restored within a few days. The main problem is that the symptoms mentioned above are quite similar for these three infections, as well as for several problems arising from allergies or physical factors, so that diagnosing the correct cause is a prerequisite for assignment of the right remedy.

Amsel et al. [4] proposed the widely used procedure (reference method) for diagnosing bacterial vaginosis (BV) that involves a series of four tests:

(i) Elevated pH level of vaginal fluid—pH > 4.5 (healthy status pH: 3.8–4.2);
(ii) Observation of clue cells and reduction in the number of lactobacilli in a direct microscopic examination after addition of a drop of saline solution to the discharge sample;
(iii) Vaginal discharge appears to be milky homogeneous;
(iv) Release of a fishy odor by addition of 10% KOH to the vaginal discharge (positive Whiff test).

The presence of three out of the four above criteria is usually required for diagnosing a patient as BV-positive.

An additional test for diagnosis of BV is based on a Gram stain of a sample of vaginal fluid. The scoring, according to Nugent, is on a scale of 0–10, where 0–3 is considered BV-negative, 7–10 BV-positive and 4–6 intermediate [5].

The fishy odor associated with BV was attributed to volatile amines, dimethylamine [6] and trimethylamine [7], found in the vaginal discharge. The presence of less volatile amines in the vaginal fluid was also reported [8–10]. Diamines (putrescine and cadaverine) were determined by an amperometric sensor [8], while gas chromatography [9] and GC-MS [10] were used to measure some other amines. The latter reported that high levels of biogenic amines (particularly putrescine, cadaverine, and tyramine) occurred in vaginal fluid samples from women with Nugent scores between 7 and 10, but were found only in very low concentrations in samples from women with Nugent scores of 0–3. Patents for diagnosing vaginal infections on the basis of measuring polyamines in vaginal fluid [11] or nucleic acid probes [12], that may serve as the basis for diagnostic kits, have been awarded.

Several IMS studies of amines [13], diamines [14] and polyamines [15] have shown that they readily form positive ions under atmospheric pressure ionization conditions and that the dominant species is the quasi-molecular protonated molecule. Thus, the gas-phase ion chemistry is controlled mainly by the proton affinities (PA) of the compounds involved. Use of n-nonylamine as reagent (PA = 219 kcal/mol) eliminates ions from compounds with lower PAs, while ion from compounds with higher PAs are selectively formed. Therefore, the direct determination of biogenic amines in vaginal discharge is made possible.

The work presented here shows that bacterial vaginosis is easily diagnosed by measurement of biogenic amines, and particularly trimethylamine (TMA), by ion mobility spectrometry (IMS) and that the presence of elevated levels of putrescine and cadaverine, with normal TMA levels, is indicative of other types of vaginal infections. Furthermore, women presenting vaginitis symptoms (pain, itching, irritation and a burning sensation) but not elevated levels normal biogenic amines, probably do not have a vaginal infection.

2. Materials and methods

2.1. Instrumentation and apparatus

The work described here was carried out with a Rotem Industries (Beer-Sheva, Israel) prototype IMS equipped with a 63Ni ionization source and heated to 130 °C, described in detail previously [16]. The sample introduction system was modified so that vapors emanating from the sample were carried by a stream of air (200–300 ml/min) into the ionization region. This carrier stream was doped with vapors of the reagent (n-nonylamine) with concentration in the ppm range, that were released from a reservoir tube. The predominant ion in the background mobility spectrum was protonated nonylamine \((K_o = 1.39 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1})\) with traces of some impurity at \(K_o = 1.63 \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}\). The drift gas flow (200–300 ml/min) was introduced, as usual, from the collector side of the drift tube.

The sample, a cotton swab with vaginal discharge fluid, collected by the gynecologist during a routine examination, was placed in a disposable 60 ml
polystyrene vial and rinsed with a 0.3 ml of de-ionized water. The swab was removed and six drops of 4N KOH solution were added, and the vial was quickly connected to the introduction system. The vapors of TMA are swept by the carrier gas stream and arrive at the drift tube within 5–10 s. In order to enhance the release of less volatile amines, and drive away the vapors of the volatile compounds that may interfere with their ionization, the vial was heated so that the sample reached a temperature of 50 °C within about 40 s. It was empirically found that addition of an acid, usually two drops of 10% nitric acid, further helps the emanation of semi-volatile amines. The vapors from the semi-volatile amines reach the drift tube 20–35 s after the beginning of the measurement. Thus, a separation in arrival time between volatile and semi-volatile amines is achieved.

2.2. Methodology

Sample collection—210 Samples (cotton tipped swabs) were collected from 129 different women during the 6 months study period (July 2001–January 2002) by the gynecologist at his hospital outpatient clinic that specializes in diagnosis and treatment of vaginitis. Each sample represents a different visit, mostly a single visit by each patient, but with some patients having up to seven visits during the study period. The patients were diagnosed by the gynecologist according to clinical symptoms and patient history as well as by vaginal discharge examination according to the reference method (Amsel test). A duplicate swab was placed in a sealed test tube and stored in a refrigerator at 5 °C. The samples were numbered sequentially in the order that they arrived at the IMS laboratory. In order to follow the efficacy of the treatment and correctness of the diagnosis, each sample had the patient’s identification number so that repeat visits by patients could be monitored. The samples were collected once a week and delivered to the IMS laboratory. In order to follow the procedure described below. Preliminary studies showed that the storage did not affect the IMS results. The IMS operators were not told beforehand whether the sample was from a BV-negative or BV-positive patients. In some cases, microbiological culture growth tests were carried out to verify the existence of trichomoniasis, candidiasis or other vaginal infections.

2.3. Calibration and data processing

Qualitative calibration of the system’s response was carried out with mixtures of aqueous solutions containing trimethylamine, putrescine and cadaverine “cocktail”, typically with 1:25:25 mol/l, respectively. Thus, 100 μl of this cocktail containing 12, 220 and 255 ng of TMA, putrescine and cadaverine, respectively, were pipetted onto a cotton swab and the procedure described in Section 2.1 for sample introduction was followed. Quantitative calibration for TMA was carried out by introducing different amounts of TMA (2–500 ng) in solution and measuring the area of the TMA peak, as described previously [17]. Quantities of TMA as small as a 2 ng per sample could thus be measured. Due their lower volatility, the detection limit for putrescine and cadaverine was about 100 ng per sample. The stability of the drift time for a given ion is within 0.1 ms and quantitative reproducibility for measurement of “cocktails” was around 15%.

Data acquisition and processing—During the measurement period (up to 90 s), a mobility spectrum (actually, average of 4 spectra) is acquired and stored every second by a dedicated software package. Each mobility spectrum may be displayed separately and the change in the intensity during the analysis for five ions (similar to single ion monitoring in GC–MS) may also be displayed. A set of empirical “expert rules”, based on a library of mobility spectra and experience, gives the operator a recommended diagnosis automatically. The physician may compare this suggested diagnosis with his own diagnosis that is based on the clinical symptoms, patient history and physical examination of the patient.

3. Results and discussion

3.1. Format of results

Fig. 1 depicts the spectra from a typical measurement: top left frame shows the mobility spectrum at a given time and the other five frames show the traces for the five ions of interest: TMA, putrescine, cadaverine, spermidine (with spermine) and n-nonylamine. As this sample was collected from a patient with BV the amine content is high. Note that the volatile TMA
Fig. 1. Measurement of a sample from a patient with bacterial vaginosis: top left frame—the mobility spectrum; Middle and lower left—the traces for trimethylamine (TMA) at 6.6 ms and n-nonylamine at 10.9 ms; Right side from top to bottom: the traces for putrescine at 7.6 ms, cadaverine at 8.2 ms, spermidine (with spermine) at 9.2 ms, respectively.

The peak at 6.6 ms enters the drift tube after a few seconds and, due to its volatility, reaches its maximum intensity within 15 s, while the semi-volatile putrescine and cadaverine (peaks at 7.6 and 8.2 ms, respectively) take a few more seconds to do so. The formation of the analyte ions is accompanied by a decrease in the reactant ion (n-nonylamine at 10.9 ms) intensity, as usually occurs in IMS.

Fig. 2 shows three of mobility spectra acquired during the measurement and demonstrates the dynamic changes in the mobility spectra measured 1–3, 11–13 and 51–53 s after initiation of the measurement (each line is the average of three spectra) in a sample collected from a patient with bacterial vaginosis. The solid line shows the background mobility spectrum during the first 3 s of the measurement where the biogenic amines from the sample have not yet entered the drift tube and the reactant ion (nonylamine) is still dominant. The dashed line shows the spectrum 11–13 s after the initiation of the measurement, where the volatile TMA is dominant and some cadaverine is also observed, while the reactant ion peak has decreased significantly. The dotted line is the spectrum recorded 51–53 s after the initiation where most of the volatile TMA has already been carried away and the semi-volatile amines (putrescine and cadaverine) are now the main peaks, while the reactant ion peak has been further diminished. The peak at 14.2 ms is due to an unidentified impurity that was present in the background.
Fig. 2. Mobility spectra measured 1–3 (solid line), 11–13 (dashed line) and 51–53 (dotted line) seconds after initiation (each line is the average of three spectra) in a sample collected from a patient with bacterial vaginosis. Note the changes in the background spectrum (solid) as volatile trimethylamine reached the drift tube (dashed) and later as the semi-volatile amines became dominant (dotted line).

3.2. Table of expert rules

The recorded parameters of the measurement included: the maximum of TMA intensity, the background levels and maximum values of putrescine, cadaverine and spermidine/spermine peaks, and the maximum and minimum of the n-nonylamine intensity. The time each product ion reached its intensity maximum was also noted. All these parameters were the basis for the analysis of the results and for the table of expert rules that provided the automatic diagnosis. Basically, when the intensity of all biogenic amines was low, and the decrease in the reactant ion peak was small, the conclusion was that the patient did not have a vaginal infection. When the level of TMA was high, and elevated levels of putrescine and cadaverine were observed, concomitant with a large decrease in the reactant ion peak then BV was diagnosed. When elevated levels of putrescine and cadaverine were observed as well as a decrease in the nonylamine intensity, but little or no TMA, then an infection other than BV was determined. Similarly, when the spermidine/spermine peak was high then an infection, other than BV, was inferred.

3.3. Diagnosis of swabs from patients

Each swab that contained a sample of vaginal fluid was measured according to the procedure described in Section 2.1 above. Of the 210 samples, 27 (12.9%) were diagnosed by the gynecologist as BV-positive and 183 as BV-negative. The automatic diagnosis, based on the mobility spectra that were stored and derived from a table of empirical expert rules, could be displayed on screen or printed.

Fig. 3 depicts the mobility spectra of swabs collected from two patients: a healthy woman (BV-negative) and a patient diagnosed by the gynecologist as having bacterial vaginosis (BV-positive). Two traces for each sample are shown: one trace acquired about
10 s after the beginning of the measurement (cold sample after addition of KOH solution) and a second trace acquired about 40 s later (heated sample and after the acid was added) showing the semi-volatile amines. The difference between the samples of the healthy and infected patients is clearly seen both in the cold spectra (a large TMA peak evident in the BV-positive sample) and mobility spectra from heated sample (large putrescine and cadaverine in BV-positive). Note also that the reactant ion peak (nonylamine) is diminished in the spectra of the BV-positive sample.

Fig. 4 shows a summary of the measurement of the 210 samples of the present study, based on principal component analysis (PCA) [18]. The circles and triangles denote the samples that were diagnosed by the gynecologist as BV-positive and as BV-negative, respectively. The X-axis and Y-axis show the first (PC1) and second (PC2) principal components, respectively. The major parameters in these components are the TMA and putrescine intensities, and the decrease in the reactant ion peak when the sample contains biogenic amines. The data points that have negative PC1 values and close to zero PC2 values belong to healthy patients, i.e., samples with low levels of the biogenic amines. Positive values in both PC1 and PC2 are indicative of bacterial vaginosis. Negative PC2 values with positive PC1 values show that the patient may be BV-negative but has some other type of vaginal infection, like trichomoniasis or candidiasis. The further a data point is from the origin of the axes the more severe is the infection. According to the Amsel test, in some cases verified by microbiological culture growth tests, these women had trichomoniasis or an advanced (heavy colonization) yeast infection. Points that are close to the origin of the axes but positive in PC1 and PC2 indicate incomplete BV. This condition would be found either when the patient is recovering from a BV infection or when the symptoms are not yet fully
developed. Similarly, points that have slightly positive PC1 (below 0.05) and small negative PC2 values (between 0 and −0.05) are indicative of a relatively mild case of trichomoniasis or candidiasis, while larger values show a fully developed infection.

3.4. Apparent false diagnoses and overall accuracy

Apparent False Negatives—Some cases appear to be misdiagnosed by the IMS, most notably five cases out of the 27 that were BV-positive according to the gynecologist but BV-negative in the IMS test (apparent False Negative). These are the data points denoted by the circles that have PC1 value above 0.05 and PC2 values below −0.1. Examination of the patients’ records and histories shows that three of these were collected from the same woman (patient X) on three different occasions a few weeks apart and were subsequently diagnosed in a microbiological laboratory culture growth test as positive for trichomoniasis. Furthermore, another of these apparent False Negatives samples was collected from a woman who was also diagnosed as positive for trichomoniasis in a microbiological laboratory and who happened to be related by marriage to patient X. Indeed, these two women shared the same husband (who also had a third wife) who was probably the carrier that transferred trichomoniasis from one of his wives to the other, so even if one was treated and cured she was re-infected by her husband. Once the whole happy family (patient X, her husband and his two other wives) was simultaneously treated, the symptoms of trichomoniasis disappeared. In the IMS study of these four cases, TMA levels were low, excluding the diagnosis of BV, but showing elevated levels of putrescine and cadaverine, compatible with trichomoniasis. There is no explanation for the fifth case that presented with symptoms of classical BV but had low TMA levels in the IMS test, so it may be regarded as a real False Negative. In conclusion, after this analysis, there seem to be only 23 true cases of BV (10.9%) of which 22 were correctly diagnosed by IMS (95.6% sensitivity).

False Positives—Only one case out of 183 diagnosed as BV-negative by the gynecologist appeared
to be marked BV-positive in the IMS test (Fig. 4, at $X = 0.08$ and $Y = 0.18$). This sample was collected from a patient who was examined on four different occasions and found to be healthy in the clinical examination in all, but this once recorded a high level of TMA in the IMS test. Three other cases had very low PC2 positive values (below 0.02) and positive PC1 values and can be considered as borderline cases for BV infection. In summary, 182 out of 183 samples were true negative giving a specificity of 99.5% for the IMS test.

Accuracy—When assessing the accuracy of the IMS test—95.1% according to the data presented in this study, one has to compare its success rate to that obtained by an expert gynecologist who meticulously performed the "gold standard" test. The IMS results, supported by culture growth tests, show that the physician marked four trichomoniasis cases as BV-positive (False Positive). The two cases of incomplete BV that were marked as BV-negative by the gynecologist provide evidence for the sensitivity of the IMS method.

4. Summary

The results presented above demonstrate that a rapid and accurate method for diagnosing bacterial vaginosis, based on measurement of biogenic amines present in a sample of vaginal fluid, was developed. IMS technology proved to be highly suitable for the measurement, as it provides the required low rates of False Negative and False Positive responses, i.e. high sensitivity and specificity. Biological samples are very complex and may contain several different chemicals, a fact that traditionally hindered the use of IMS for biological applications and medical diagnostics in such matrices. However, control of the ion chemistry with a suitable reagent (n-nonylamine) and use of a simple, yet resilient, sample introduction system that helps separate the volatile from the semi-volatile compounds, enabled us to overcome these problems. The use of a dedicated software package for acquisition, storage and processing of the mobility spectra relieves the physician from the burden of understanding the intricacies of IMS technology and allows the doctors to confirm their own diagnosis. The dynamic changes during the measurement provide further indications regarding the nature of the biogenic amines present in the sample.

The method can easily differentiate between healthy women who do not have a vaginal infection (and therefore only background levels of biogenic amines) and women who have some form of vaginitis. The present work demonstrated that the IMS method can be used with an accuracy of over 95% to diagnose bacterial vaginosis, a widely spread and common form of vaginitis that is often misdiagnosed by standard methods. As for trichomoniasis, that is frequently confused with BV in the Amsel test (both have elevated pH values and malodorous discharge) even though microscopy should be able to distinguish between the two, the IMS clearly shows when BV is present (large TMA signal).

The results unequivocally show that elevated levels of biogenic amines are present in samples of vaginal fluid collected from BV-positive women, and a direct correlation was found between elevated TMA levels and BV. This is in agreement with most published reports. Measurement of the total amount of amines, even in combination with pH determination, is insufficient for diagnosis of BV, as elevated levels of putrescine and cadaverine are found in samples collected from BV-negative women with trichomoniasis or advanced candidiasis. The differentiation between volatile and semi-volatile amines may somewhat reduce the error level, but the advantage of direct measurement of each amine, as provided by IMS, was clearly shown in this work. We are currently involved in clarifying the role and relation of the semi-volatile amines in particular types of infections.

References