Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 4 [4] March 2015: 43-46

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Correlation between Age and Histological Changes of Pap Smear Lam Microscopy

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ABSTRACT
Vaginal douching is the process of intravaginal cleansing with a liquid solution. Douching is used for personal hygiene or aesthetic reasons, for preventing or treating an infection, to cleanse after menstruation or sex, and to prevent pregnancy. Cluster sample were collected in AliNasab hospital Department of Pathology patients pathology with age from 19 to 53 years Provided that the total of 94 patients and 11 samples from each patient were available slides. Samples immediately after sampling were fixed with ethanol, methanol and acetone and transported to the laboratory According to Sigma - Aldrich Cytochemical staining was performed. There was a statistically significant correlation between the quality of microscopy in detecting signs correlation the presence of the dominant bacterial population is determined as indicated in 35 - 45 years had the highest diagnostic quality, then aged 25 - 35 age group and then15-25 age with more than 85% difference in laboratory studies to provide the necessary competence. In the samples obtained in the age of 50 -45 the maximum rate is 55%. This method of Review is less than 45 years is recommended (Z: - 2.201, Sig: 0.028).
Keywords: Correlation, Vaginal, Histological changes, Pap Lam microscopy, Iran

Received 12.12.2014 Revised 03.01.2015 Accepted 19.01.2015

INTRODUCTION

Vginal discharge is a common presenting symptom and may be either physiological or pathological. The mostcommon causes of vaginal discharge are physiological, bacterial vaginosis and candidal infections [1]. Sexually transmitted diseases (STDs) and non-infective causes need consideration also. A normal physiological discharge is a white or clear, non-offensive discharge that varies with the menstrual cycle [2]. The perception of signs and symptoms at the level of the lower genital tract is the most frequent cause of medical visits of pregnant and non-pregnant women in fertile age, and with minor degree, but significant importance in menopausal women. In addition a consider able number of women make non-medical consultations in pharmacies and another even higher number of women bear their condition either without any treatment or using "home" treatments. The morphological study of the Balance of Vaginal Content (Balance delContenido Vaginal: BACOVA in Spanish) shows that up to 50% of pregnant and non-pregnant asymptomatic women present significant alterations in at least one of two basic vaginal functions. A significant percentage (15 to 30%) of women who consult for symptoms (in the absence of obvious signs) of vaginal itching, burning sensation, or dyspareunia, show no morphological alterations in the vaginal content [1]. The great magnitude of the problem of VD, which includes symptomatic and asymptomatic patients, but all exposed to an increase in sexual and reproductive health risks, requires a joint effort of the biomedical staff, healthcare providers, organized social groups and the National Health Service, to optimize the primary attention of vaginosis/vaginitis in women in fertile age and menopause. The first stage is to improve and coordinate the diagnosis of VD. The signs and symptoms compatible with VD, are presented individually or associated in an arbitrary and disjointed manner. The most frequent are: itching, burning sensation, irritation, odor, abnormal vaginal discharge, edema in the vulvo vaginal area, vulvodynia, dysuria, dyspareunia and/or pain in the pelvic region. The signs and symptoms mentioned above, either individually or associated with a significant number of pathologies of the female genital tract. They are not pathognomonic for diagnosis of vaginosis, vaginitis or any specific etiology. They only allow establishing, in a presumptive way, the state of possible VD [1, 2]. Multiple studies conclude that a reliable

diagnosis cannot be made on the basis of history or physical examination along [1, 11] .A very efficient laboratory study has been developed. [1-7].The morphological study of the BACOVA (Annex I), which integrates two basic vaginal functions evaluation. The systemic regulation of the vaginal microbiota using Nugent score (NS) [5] and the simultaneous determination of the Vaginal Inflammatory Response (VIR) (8), allow the precise diagnosis of the two most [2]. Highly frequent vaginal pathologies: Vaginosis and Vaginitis and with a high predictive value, cases in which both alterations are present [1]. RIV is established with a high efficiency counting procedure for leucocytes in de vaginal content. Vaginosis is defined, based on the alteration of the healthy vaginal ecosystem and demonstrated absence of VIR [3, 5, 9]. Vaginitis shows presence of significant VIR in the vaginal content, with or without alterations of the healthy vaginal ecosystem (1, 6, and 9).Vaginitis, with or without simultaneous vaginosis. The etiology of vaginosis is not definitely clear (10). However, there is agreement that the metabolic factors include a systemic imbalance of the "estrogen factor" and/or an alteration in the innate proinflammatory response as a stage previous to the alterations in the complex function (sexual/reproductive) of the vagina [1]. Till now the most conspicuous detection of these alterations is the change in the balance of the healthy vaginal ecosystem. A decrease in the relative amount of Lactobacilli and a simultaneous increase in the endogenous anaerobic microbiota [1, 5, 9, 10]. Internationally accepted bacterial morphotypes count proposal by Nugent [5] is the gold standard [7]. Nugent Score have been incorporated in BACOVA, but there are others variations of the same basic methodology with valid results [12, 13]. In addition to induce the reduction lactobacilli and promoting the relative growth of the native anaerobic microbiota of the vagina, the state of vaginosis or primary vaginal dys function sign ificantly increases the colonization of opportunistic bacteria in the vaginal content of all women in fertile age/menopausal, independently of whether they are sexually active or not. At the same time, it increases the risk of acquiring and transmits sexually transmitted infections (STIs) in those who are sexually active (1). So far, no specific infectious etiology of vaginosis has been found (10). Common bacteria of the vaginal content, such as *Gardnerella vaginalis, Atopobium vaginae, Mycoplama spp., Ureaplasma spp., Prevotellaspp.,Clostridiumspp., Leptotrichiaspp., Megasphaera* spp., and many others (1), can become aggressive depending on the degree of the systemic alterations manifested in the function of the vaginal epithelium. The increase in the relative amount of these species and their eventual aggressiveness depends on the insufficiency of the epithelium previously damaged by the systemic primary vaginal dysfunction. The ability of some of these bacteria to develop biofilms has added important material of controversy in the discussion of the problem of relapses in patients with Bacterial Vaginosis [1, 12]. Infact, until the present, no etiologic factor of Bacterial Vaginosis can be assigned to a specific microorganism. This have been confirmed with the recent research of the microbiome of vaginal content, in which 280 Operational Taxonomic Units (OTUS), including a significant number of no cultivated species, have been identified [1]. The controversial results of antibiotic treatment could be explained accepting that the real etiology of BV is not infective. Vaginitis as a basic diagnostic evidence, requires the presence of VIR in the vaginal content, and the etiology could or could not be infectious. As exceptions, with very low frequency in fertile age, VIR could be associated with atrophic non-infectious vaginitis [3]. Its significant increase of leucocytes in the vaginal content is a strong sign of the vaginal, cervical and/or upper urogenital infection. There are few specific infectious agents that produce real vaginitis, but two of them, Yeast and Trichomonas, have a universal and high prevalence.

MATERIALS AND METHODS

Sterile speculum, sterilized cotton swab, slide glass, diamond pen, ethanol, methanol, All of absolute acetone, Kits of Heat stain, Giemsa, Ziehl-Neelsen, blue Toluidine Also Chloroethyl acetate AS-D kits myeloperoxidase, Sudan black B, Alpha- naphthalenpropanoic acetate esterase, acid phosphatase, Periodic acid-Schiff all manufactured by Sigma - Aldrich, production of Germany. The results of the Papanicolaou staining in the Tabriz Ali NasabEram Hospital on the same samples were performed. Olympus microscope with magnification imaging companyX100.

Methods

Sampling

By information from hospital documents of Tabriz in the sampling Pap smear and through carried out former coordination to hospital admission, and women who, because of problems Vaginal Pap test were referred to hospital in the age category 19 to 55 years of sample were collected. Attending random cluster sampling from each patient11 slides of vaginal mucous samples were taken immediately after sampling and drying slides Fixation solution, all samples were fixed.

Preparation of samples and vaginal smear:

Vaginal mucosa samples were taken from each of 11 slides By cotton swabs and slides all of by The dried slides were coded by diamond pen temporary solution Fixation unit for 1 share as ethanol, methanol and 3

share 1 share of acetone was prepared and then fixed in the box Preparation of documentation and the Laboratory of Immunology we've transferred Lahijan Azad University. In order to Evaluation of the infection of 1 Lam of each sample was Papanicolaou staining. The slides were stained for confirmation the presence of Normal bacterial flora of bacteria and detection of Heat.In order to confirm the diagnosis of fungus infections of Lam Papa Nicolas one Lam from each sample Toluene were painted by blue color. Ziehl-Neelsen staining for the detection. In the Next to detect protozoan normal flora as well as the pattern of leukocyte one Lam The slides were stained with Giemsa stain series of data based on cell morphology White, staining the white blood cells in the population are considered to be preliminary So if there is a disruption in the collection of samples, so Evaluation of white blood cells Influence is clarified.

In order to Evaluation of the differential diagnosis of mature cells based on Lukugram Classic 5 ID epithelial cells made , white blood cell patterns, coloring Cytochemical Chloroethyl esterase, Alpha-naphthalene propanoic\ Phosphatase acetate esterase, acid-AS-D myeloperoxidase, Sudan black B, Naftul Phosphatase, Periodic acid-Schiff using commercial kits manufactured by Sigma-Aldrich .

Staining

Heat staining method:

First slides put on the tray with a solution of 1 volume of ethanol, 1 volume of methanol and 3 volumes of acetone-fixed and after Transfer to the prepared slides laboratory staining. A few drops of crystal Violets slides and throw on lams for about a minute then we wait, during this time all bacteria will be purple. After washing with water, juice, crystal violet by adding Logol are established for a minute. Heat crystal violet combined creation Complexes above the crystal violet dye fixing inside the bacterial cell wall A. After this step, all the bacteria continue to appear purple. After this step washed Lam with water. Then decolonization that is the most important step of staining has done.

Review of stained expansion:

Because the needs of all groups of leukocyte cells and mature properly and accurately and with The ability to separate from one another very much known appearance of white blood cell morphology The expansion of the painted colors and patterns observed in cytochemical staining was compared Rumanufsky case and extensive pattern recognition penta valent White blood cells were obtained. Expansion which features a stained expansion not appropriate was excluded in the Review sample.

Assess the suitability of specimens for Pap tests:

Assess the suitability of the sample to be tested by staining by Papa Nicolas The pathologist examined and the results are divided into three categories, namely within Epithelial beginning cellular changes , see descriptive diagnosis normal limits cell abnormality , see descriptive diagnosis.

Pathogen detection

Lam stained with Papanicolaou colors, heating, Giemsa, blue toluidine and acid periodic. Schiff were included to identify the pathogen and normal flora diagnosis. Review slides Olympus microscope imaging done on the basis of Heat stain morphology *cocobacill* and heat-positive bacteria and negative, yeast and protozoa observed. With purple bacteria are heat positive and heat negative part is pink. It also changes with leukocyte cells in the vaginal area and observed epithelial cells rather than study for detection of viruses and bacteria. Therefore intracellular buildup of both subspecifictaxa was divided into 4 categories causative factors which are respectively viral, Protozoan, Bacterial, Fungal.

Changes in cell morphology

All changes in appearance of including changes in inflammatory cells, epithelial cells, abnormal, Operative changes acute or chronic infection Papanicolaou staining were examined and which were classified in the following way. The response of tissue cells into three categories: low, moderate, and severe tissue reactions were divided into three categories: Typical repair, Follicular cervicitis and Atrophic vaginitis. Abnormalities of the epithelial cells are divided in three categories: Atypical squamous cells of undetermined significance, High or low grade squamous intraepithelial lesion and Invasive squamous cell carcinoma .also, the percentage of people was examined who have an acute inflammation.

RESULTS AND DISCUSSION

There was a statistically significant correlation between the quality of microscopy in detecting signs correlation the presence of the dominant bacterial population is determined as indicated in 35 - 45 years had the highest diagnostic quality, then aged 25 - 35 age group and then15-25 age with more than 85% difference in laboratory studies to provide the necessary competence. In the samples obtained in the age of 50 -45 the maximum rate is 55%. This method of Review is less than 45 years is recommended (Z: -2.201, Sig: 0.028).Because there are significant differences between the variables identified protozoal agent and the relationship with age, patient can be realized by way of any of the age categories studied microscopy It will not suffice to identify protozoal agent and review these practices in order So reliable but is not recommended as the primary cause symptoms cervicovaginal clinical population study of fungi,

bacteria and viruses (Z: - 2.521, Sig: 0.012).According to the statistical correlation between involvement confirmation viral infectious agent causing inflammation and infection in the area, especially in the age group 25 to 35 years Cervico-vaginal And After that at age categories 15 -25 and 35-45 years and 55-45 in the last place Since it is clear that the application of this method and intervention microscopy in detecting and identifying viral efficiency are necessary (Z: - 2.934, Sig: 0.003). Due to the transition of virginity at the age of 15-25 years or experience in health and use of chemicals or pharmaceuticals advantage of tracking changes in inflammatory cells indicates that more basophilic Inflammatory responses are sensitive to nature The period between the ages of 25-35 and then 45-55 years and 35-45 years of age with a minimum incidence of microscopic attention to quality can be Said that because of pregnancy and lactation periods of 25-45 years of age and a significant reduction in clinical infections, cervico-vaginal and On the other hand the devastating hormonal changes at the age of 45-55 years lower incidence of allergic inflammatory responses can be expected. It Should not be forgotten that the recommendations of Physical and hormonal methods of prevention or preventive physical and chemical methods are combined At the age of marriage, especially after several successful pregnancies have been asked and that happens more aged 25 -45 years can cause serious allergic reactions relative reduction in the area under study. Immunological tolerance and catering can also be involved in reducing the serious consequences (Z: - 2.448, Sig: 0.014).Severe inflammation and tissue reactions are always visible in microscopic that decrease with increasing age. So, at the age of 15-25 years, 45-55 years, and severe inflammation prevalence is much higher than the Middle Ages (Z: - 2.158, Sig: 0.031). Tissue reaction significantly due to microscopic protozoan cause inflammation in this manner is not verifiable (Z: -3.061, Sig: 0.002). According to the statistical correlation between the level of histological changes and the presence of protozoa as Normal flora can be Said no relation to the involvement of protozoa agent and histological changes in the by microscopy (Z: - 2.521, Sig: 0.012). According to the statistical correlation between changes in epithelial cells and protozoa agent, incompetence protozoa infections and detection changes in epithelial made with microscopic method (Z: - 2.366, Sig: 0.018). Due to these infectious agents that were statistically significant at all ages of Activated neutrophils core Segmented always accompanied despite inflammatory response, acute non-specific cell type, healing ability and then follicular cervicitis are more common. The presence of neutrophils. Bound increases with age, but more can be done to reduce the severity of neutrophils Segmented increases with age (Z: - 2.134, Sig: 0.033).

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CITATION OF THIS ARTICLE

Morvarid M, Omid L A and Maryam I. Correlation between Age and Histological Changes of Pap Smear Lam Microscopy . Bull. Env.Pharmacol. Life Sci., Vol 4 [4] March 2015: 43-46