RESEARCH ARTICLE

Editorial Process: Submission:06/09/2022 Acceptance:03/09/2023

Evaluation of 1,030,482 Cervical Smear Results in Brazilian Population

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Abstract

Background: The objective was to determine the prevalence of abnormal cytologic smears in a large population of a Brazilian city. **Methods:** Retrospective study of cervical cytology results performed at a private laboratory in São Paulo - Brazil. A total of 1,030,482 cytology tests were performed between January 2010 and December 2015. **Results:** Among the satisfactory cytologies, we observed abnormal results in 8.9% (91,371). Analyzing the proportion of exams with altered results over the years, there was no change in these rates. We observed that the proportion of abnormal exams decreased with increasing age. Atypical squamous cells of undetermined significance, possibly non-neoplastic (ASC-US) cytology was the most common abnormality found in the general population with 73.19%, followed by low-grade intraepithelial lesion (LSIL) with 20.5%; these 2 cytological abnormalities add up to 93.69 % of all abnormal results. There was an increase in ASC-US with advancing age, a decrease in prevalence of LSIL, especially after the age of 30 and high-grade intraepithelial lesion (HSIL) shows two peaks of elevation, respectively in the age groups of 30-39 years and over 70 years. **Conclusion:** Women under the age of 30 showed a higher prevalence of cytological abnormalities, however most of these changes are low-grade lesions. In older women, cytological abnormalities are largely high-grade lesions, requiring greater care to prevent progression to cancer.

Keywords: Cervical cancer- cervical intraepithelial neoplasia- cytological techniques- early detection of cancer

Asian Pac J Cancer Prev, **24 (3)**, 867-872

Introduction

Cervical cancer is the second most common cancer and the third most important cause of cancer mortality in women worldwide. Worldwide, cervical cancer was responsible for more than 265 thousand deaths in women in 2012, with 87% of these deaths occurring in developing countries (Isaoglu et al., 2015). In our country Brazil, this tumor occupies the third position in number of cases of female cancer. The number of new cases of cervical cancer expected for Brazil for each year alone in the 2020-2022 period is 16,590, with an estimated risk of 15.43 cases per 100,000 women (Ministry of Health of Brazil, 2016).

The most important risk factor for the development of cervical cancer is the presence of the human papillomavirus (HPV), with its oncogenic subtypes. Studies in the literature have shown that more than 97% of cervical tumors contain HPV DNA (de Sanjose et al., 2010). Currently, screening of this tumor is performed through the cervical cytology and / or HPV DNA test; the use of these two tests (co-testing) is advocated by some international organizations (Saslow et al., 2012; Sauer et al., 2015). However, according to the Brazilian Ministry of Health, cervical cancer screening should be performed by cytological examination. Also in other developing countries, cytology is still the screening test for cervical cancer. Recommendations in Brazil are to start screening at age of 25 in women who have already started sexual activity. After two negative exams, performed one year apart, the next ones must be performed every 3 years, up to 64 years old. The predominant pattern of screening in Brazil is opportunistic, or in other words, women have had a Pap test during a routine medical consultation or a consultation for an unrelated condition (Ministry of Health of Brazil, 2016).

The Nomenclature currently used in Brazil is the Brazilian Nomenclature for Cytopathology Reports (NBLC) (Ministry of Health of Brazil, 2006), which was based on the Bethesda System and incorporated by the laboratories that provide services to the National Program for Cervical Cancer Control.

However, there is no complete and accessible official data on the prevalence of cytologic abnormalities in our country. The data collected by the computerized system show only the population that uses the public health system, and these data are presented in an incomplete way. Thus, it would be important to obtain these data to assess the general status of the abnormalities in order

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to better develop and implement a national screening program that is effective for the prevention of cervical cancer. Furthermore, it is interesting to disclose these data, so that other institutions around the world can compare their cytological findings with those presented here. Thus, the objective of the present study was to determine the prevalence of abnormal cytologic smears in a large population of the largest city in Brazil, that is São Paulo.

Materials and Methods

This is a retrospective, cross-sectional study carried out by surveying the results of all cervical-vaginal cytology smears performed at a private laboratory in São Paulo city – SalomãoZoppi Diagnósticos (Brazil), between January 2010 and December 2015. The data were obtained from the Data Processing Center of the institution. This study was approved by the Research Ethics Committee of the institution (CAAE 64365817.0.0000.5479) and was exempted from using a Voluntary and Informed Consent Form (VICF) since it was a retrospective study involving the analysis of the institution's database. Ayre spatula and cytobrush were used for the collection of cytological exams and they were collected by gynecologists or by nurses.

It is known that some patients need semi-annual exams, according to the recommendations of the Ministry of Health of our country. Thus, in order to evaluate the repetition of exams by the same patient, we reviewed the registration numbers of each woman included in the study. Of the total number of tests, only 426 patients (0.04% of the sample) underwent more than one test in the same year, with no patient having performed more than three tests during the period evaluated. Therefore, these exams were not excluded.

We excluded all unsatisfactory tests, i.e. those tests where the cytopathologists were unable to assess the findings. For each patient, the following data were available: patient code, test request code, age, date of the test (month and year) and cytopathology test result (Bethesda criteria). Due to the fact that it is a database, we are unable to access the patients' medical records, as well as their personal data.

The reports of cytological exams were issued according to the 2001 Bethesda system(7) into: negative for neoplasia (no cellular abnormality); ASC-US (atypical squamous cells of undetermined significance, possibly non-neoplastic); ASC-H (atypical squamous cells, cannot exclude HSIL); AGC (atypical glandular cells); LSIL (low-grade intraepithelial lesion); HSIL (high grade intraepithelial lesion); invasive SCC (squamous cell carcinoma); AIS (adenocarcinoma in situ); invasive adenocarcinoma; invasive endometrial adenocarcinoma and HSIL cannot exclude microinvasion.

The data obtained were tabulated in a Microsoft Excel® spreadsheet and subsequently exported to the SPSS Statistics® software package. The association among variables of interest was established using the Chi-square test; we adopted a level of significance of 0.05. A linear regression curve was plotted to correlate variables with year and the R^2 calculation was employed to measure model fit.

Results

At SalomãoZoppi Diagnósticos (Brazil), between January 2010 and December 2015, 1,030,482 cytology exams were performed. Of these, 3,811 (0.36%) unsatisfactory samples (those for which the cytology could not be interpreted) were excluded, giving a final total of 1,026.671 satisfactory cytology samples.

The age of the patients ranged from 13 to 95 years, with a mean of 39.3 years ± 12.8 years (standard deviation). When dividing the patients by age groups, we found 2% with age up to 19 years; 22.1%, from 20 to 29 years old; 33.3% from 30 to 39 years; 21.3%, 40-49 years; 13.6% from 50 to 59 years; 5.6%, from 60 to 69 years; and, 2.1% aged 70 years and over.

Among the satisfactory cytologies, we observed negative results in 91.1% (935,300) and abnormal results in 8.9% (91,371), regardless of the period and age range. Analyzing the proportion of exams with altered results over the years, there was no change in these rates (Chi-square test, $p < 0.001$) (Table 1). Analysis of the linear regression curve confirmed this result, showing that rates of cytologic abnormalities did not change over time with $R^2 = 0.1067$ ($R < 0.7$ does not confirm trend).

Table 2 shows the prevalence of the abnormal cytological results, according to the total of cytologies and according to the altered cytologies. ASC-US cytology was the most common abnormality found in the general population with 73.19%, followed by LSIL with 20.5%; these 2 cytological abnormalities add up to 93.69 %.

Table 3 shows the prevalence of cytological abnormalities over the course of the study years. Analyzing the rates of the main cytological abnormalities, ie, ASC-US, LSIL, HSIL, ASC-H and AGC, the linear regression curve did not show a tendency to fall or increase these rates according to time, with all presenting $R^2 < 0.7$ (does not confirm trend).

By stratifying the patients by age group, we observed that the proportion of abnormal exams decreased with

Table 1. Prevalence of Negative and Altered Results Over the Years of the Study

Year	2010		2011		2012		2013		2014		2015	
	n	%	n	%	n	%	n	%	n	%	n	%
Altered	10.982	8.9	12.3	8.9	14.738	9.3	17.064	9.5	18.349	9.4	17.938	7.8
Negative	112.109	91.1	126.826	91.1	144.157	90.7	162.938	90.5	177.157	90.6	212.113	92.2
Total	123.091	100.0	139.126	100.0	158.895	100.0	180.002	100.0	195.506	100.0	230.051	100.0

(Chi-square test, $p < 0.001$)

Table 2. Prevalence of Cytologic Abnormalities from 2010 to 2015, According to the Total of Cytologies and According to the Altered Cytologies

Cytological abnormality	N	Among all altered results (%)	Among all samples (%)
ASC-US	66.878	73.19	6,514
LSIL	18.729	20.50	1,824
ASC-H	2.459	2.69	0.240
HSIL	1.859	2.03	0.181
AGC	1.355	1.48	0.132
HSIL-suggestive of invasion	50	0.05	0.005
Adenocarcinoma	28	0.03	0.003
SCC	13	0.01	0.001

Table 3. Prevalence of Cytologic Abnormalities Over the Years of the Study

Cytologic result	2010		2011		2012		2013		2014		2015	
	n	%	n	%	n	%	n	%	n	%	n	%
ASC-US	7.964	72.52	9.272	75.38	11.231	76.20	12.953	75.91	13.86	75.54	11.598	64.66
LSIL	2.355	21.44	2.425	19.72	2.594	17.60	3.008	17.63	3.331	18.15	5.016	27.96
ASC-H	266	2.42	267	2.17	394	2.67	504	2.95	480	2.62	548	3.05
HSIL	276	2.51	233	1.89	261	1.77	267	1.56	311	1.69	511	2.85
AGC	104	0.95	93	0.76	236	1.60	320	1.88	355	1.93	247	1.38
HSIL - suggestive of invasion	11	0.10	5	0.04	10	0.07	8	0.05	5	0.03	11	0.06
Adenocarcinoma	3	0.03	3	0.02	7	0.05	4	0.02	6	0.03	5	0.03
SCC	3	0.03	2	0.02	5	0.03	0	0.00	1	0.01	2	0.01
Total	10.982	100	12.3	100	14.738	100	17.064	100	18.349	100	17.938	100

increasing age (Table 4) (Chi-square test, p <0.001). We observed the following percentages of cytological abnormalities by age group: 16.28 % with age up to 19 years; 14.47 %, from 20 to 29 years old; 8.73 % from 30 to 39 years; 6.88 % from 40-49 years; 5.16 % from 50 to 59 years; 3.88 %, from 60 to 69 years; and, 3.96 % aged 70 years and over.

The prevalence of cytological abnormalities according to the age groups are presented in table 5. We observed a clear increase in the prevalence of ASC-US with advancing age, a decrease in prevalence of LSIL, especially after the age of 30 and an increase in ASC-H over 50 years. The AGC has two peaks of elevation, that is, in the range of 40-49 years and another peak in patients over 70 years; the HSIL shows two peaks of elevation, respectively in the age groups of 30-39 years and over 70 years.

Discussion

Cervical cytology is a simple and inexpensive test, which has been used to screen for cervical cancer and its preinvasive lesions. Cytology has been encouraged as a screening method in developing countries with a lack of resources due to its low cost and good efficacy in the detection of cervical lesions, especially high-grade lesions (Tokmak et al., 2014).

The prevalence of cytological abnormalities in our sample was 8.9%, slightly higher than the Israeli rates (4.78%), a study published in 2014 (Bassal et al., 2014) and the data collected at public hospital in Brazil, which was 5% (Henrique et al., 2017). The most prevalent cytological alterations in our total population were: ASC-US with 6.5% of the total (73.19% of the altered cytologies), LSIL with 1.82% (20.50% of the altered cytologies), ASC- H with 0.24% (2.69% of the altered cytologies), HSIL with 0.18% (2.03% of the altered cytologies) and AGC with 0.13% (1.48% of the altered cytologies). The two most commonly found cytologies were ASC-US and LSIL, with ASC-US present in 73.19% of the altered cytologies.

Stolnicu et al., (2014) showed that the respective prevalence rates of ASC-US and LSIL in Romania where were 2.6% and 0.8% and for Bassal et al., (2014) in Israel they were 2.72% and 1.54%, respectively. The prevalence

Table 4. Prevalence of Cytologic Results by Age Group (years)

Cytologic result	≤19 y		20 to 29 y		30 to 39 y		40 to 49 y		50 to 59 y		60 to 69 y		≥ 70 y	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Abnormal	3.405	16.28	32.832	14.47	29.841	8.73	15.038	6.88	7.19	5.16	2.217	3.88	837	3.96
Normal	17.435	83.72	192.562	85.53	306.334	91.27	200.004	93.12	112.04	94.84	37.079	96.12	11.884	96.04
Total	20.84	100	225.394	100	336.175	100	215.042	100	119.23	100	39.296	100	12.721	100

y, years; Chi-square test, p <0.001

Table 5. Prevalence of Cytologic Results by Age Group (Years)

Cytologic result	≤19 y		20 to 29 y		30 to 39 y		40 to 49 y		50 to 59 y		60 to 69 y		≥ 70 y	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ASC-US	2.157	63.35	22.618	68,890	22.198	74,388	11.677	77,650	5.783	80,431	1.808	81.55	627	74,910
LSIL	1.174	34.48	8.684	26,450	5.58	18,699	2.264	15,055	828	11,516	169	7.62	29	3,464
ASC-H	42	1.23	710	2,163	820	2,748	355	2,361	291	4,047	150	6.77	91	10,872
HSIL	26	0.76	688	2,096	751	2,517	256	1,702	85	1,182	32	1.44	21	2,508
AGC	6	0.18	123	0.375	462	1,548	470	3,125	193	2,684	52	2.35	49	5,854
HSIL - suggestive of invasion	0	0.00	5	0.015	19	0.064	10	0.066	3	0.042	4	0.18	9	10,752
Adenocarcinoma	0	0.00	3	0.009	6	0.020	4	0.027	6	0.084	2	0.09	7	0.836
SCC	0	0.00	1	0.003	5	0.017	2	0.013	1	0.014	0	0.00	4	0.477
Total	3.405	100	32.832	100	29.841	100	15.038	100	7.19	100	2.217	100	837	100

y, years

of invasive squamous cell carcinoma was 0.001% in our sample, compared to 0.07% in Romania (Stolnicu et al., 2014). In Turkey in 2009, the prevalence of ASCUS, ASC-H, LSIL, HSIL, and AGC was 1.07%, 0.07%, 0.3%, 0.17% and 0.08% respectively (Turkish Cervical Cancer And Cervical Cytology Research Group, 2009). We can observe that these values are lower than the reference values obtained in Latin American countries, including our country.

The higher frequency of cytologic abnormalities in our country can be explained by the higher prevalence of HPV in Latin America. Bruni et al., (2010), showed that the overall prevalence of HPV DNA was 11.7%, with data obtained from around one million cytologies on five continents. On the other hand, the authors observed that Sub-Saharan Africa (24%), Eastern Europe (21.4%) and Latin America (16.1%) showed the highest viral prevalence in the world.

When analyzing the cytological alterations in the different age groups we observed some variations in the prevalence between the groups. Although young women have a higher percentage of cytological abnormalities than older women, most of the changes found in this age group refer to low-grade lesions. Whereas, the so-called high-grade abnormalities are found more often in older patients. This fact is related to the natural history of HPV infection, where viral persistence in older age groups causes the development of precancerous lesions.

A clear increase in the prevalence of ASC-US can be observed with advancing age. This increase must be justified by the greater atrophy in older women and possible collection difficulties. It was also observed a fall in the prevalence of LSIL, mainly after 30 years; this high prevalence of low-grade lesions in women younger than 30 years can be explained by the high prevalence of HPV infection in this age group. Younger women are particularly susceptible to infection with this virus as a result of the multiplicity of sexual partners, resulting in contact with several types of virus, with a peak prevalence of HPV in women under 24 years of age (Bruni et al., 2010; Sauer et al., 2015).

In young people, biological factors such as immaturity of the cervix with physiological ectopy, associated with large areas of metaplasia, favor HPV infection at the site of the cervix transformation zone (DeCew et al., 2013). Especially in young women, most HPV infections are transient, with viral elimination in 18 to 24 months in more than 90% of cases (Sauer et al., 2015; Duke et al., 2015).

Due to the high prevalence of ASC-US and LSIL changes in young people, associated with the high index of regression of these lesions in this age group, in our country the ministry of health recommendation for these cytological abnormalities is cytologic follow-up.

The AGC presented two peaks of elevation, in the range of 40-49 years and another peak in patients over 70 years. These peaks coincide with the age ranges that this abnormality is related to. Although a cytologic abnormality is not very prevalent, it is somewhat worrying. An Australian study was conducted between 2006 and 2012 in order to evaluate the prevalence of histological abnormalities of the cervix in patients with AGC. The authors observed 12% of lesions high histological grade and AIS, 0.7% of cases of cervical cancer, 3.8% of endometrial cancer and 8.9% of lesions of low histological grade, confirming the need for specific gynecological follow-up with colposcopy and follow-up. Younger women (25-34 years of age) were more likely to develop high-grade lesions, but the 45- to 54-year-olds showed the highest risk of developing high-grade lesions in 5 years (Munro et al., 2015).

Zhao et al., (2009) observed precancerous or malignant neoplastic histological findings were documented in 15.3% of the AGC, including 8.3% cervical, 6.3% endometrial and 0.6% ovarian. The results of AGC were most often associated with neoplastic cervical outcomes in women younger than 40 years and with endometrial neoplastic outcomes in women aged 50 years or older.

On the other hand, HSIL shows two peaks of elevation, respectively in the 30-39 year and 70 year old ranges, which is justified by the natural history of HPV with the peak incidence of low grade lesions before 30 years and, with a period estimated from 10 to 15 years for lesion development, the peak incidence of high grade lesions occurs in the following decade.

The American Cancer Society (ACS) advises that women who are 70 years of age or older can choose to discontinue the Pap test if they have three or more normal tests with no change in the past 10 years. The ACOG

2009 guidelines recommend ceasing screening with the cytologic test at age 65-70 years in patients with the same prior prerogatives. An ACS update recommends that women over 65 years of age with evidence of adequate negative prior screening and no history of CIN 2 or higher within the past 20 years should not be screened for any form of cervical cancer. In Turkey, random screening is very widespread, as in Brazil, and therefore we have a large number of cytologies collected outside the age ranges of the national guidelines (Gyllensten et al., 2018). The morphological alterations of the postmenopausal cells reduce the adequacy of the cytological sample and therefore, for a more adequate screening, the HPV-DNA test in this age group should be considered.

Screening for cervical cancer using cervical-vaginal cytology is highly effective in reducing the incidence of invasive cervical cancer and death among women of childbearing age. However, screening data for older women are limited because the sensitivity of Pap smears varies with screening age (Rustagi et., 2014; Gyllensten et al., 2018). There is currently a proposal to introduce high-risk HPV DNA tests as a primary cervical neoplasm detection strategy. In the US the co-test (cervical cytology + HPV test) is preferred every 5 years in the age range of 30 to 64 years (Fontham et al., 2020).

Our study has some limitations, the main topics being: a) inclusion of data from a single health service operator and not based on the Brazilian population as a whole; b) we were unable to associate cytological abnormalities with colposcopy and histological results, when there was a need for biopsy; c) the data were based on opportunistic screening, as opposed to an organized screening program. Despite these limitations, we believe that the large number of cytological samples analyzed gives high power of analysis and strength to the conclusions of the study and these data will help physicians and epidemiologists in decision-making.

The data found show that there are patterns in the prevalence of cytological abnormalities that should be considered for the quality of population screening. Women under the age of 30 present a higher prevalence of low-grade lesions and this fact should be considered when interpreting the results in women in this age group. On the other hand, in older women, the rate of ASC-US and AGC increased, suggesting greater difficulty in reading these sheets and the need for HPV testing in this age group should be considered.

Author Contribution Statement

A) Campaner AB conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Performed the analysis; Wrote the paper. B) Fernandes GL conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Performed the analysis.

Acknowledgements

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

If it was approved by any scientific Body/ if it is part of an approved student thesis

The present study was approved by the scientific committee of the Gynecology Department institution of Santa of São Paulo Hospital - Brazil.

How the ethical issue was handled (name the ethical committee that approved the research)

This study was approved by the Research Ethics Committee of the institution Santa of São Paulo Hospital – Brazil (CAAE 64365817.0.0000.5479)

Availability of data (if apply to your research)

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Any conflict of interest

The authors report no conflict of interest."

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