раковины у 79-летнего пациента белой расы, которому проведено одноэтапное реконструктивное хирургичес-кое лечение методом модифицированного лоскута Antia-Buch. В статье обсуждаются особенности БКК в области ушной раковины и варианты реконструкции наружного уха.

რეზიუმე

ყურის ნიჟარის ერთეტაპიანი რეკონსტრუქცია ANTIA-BUCH-ის მოდიფიცირებული ნაფლეთის მეთოდით შერეული ტიპის ბაზალურ-უჯრედული კარცინომის R0-რეზექციის შემდეგ (კლინიკური შემთხვევა)

¹ა. გოლდმანი, ²უ. ვოლინა

¹გოლდმანის კლინიკა, პორტუ-ალეგრი, ბრაზილია; ²დრეზდენ-ფრიდრიხ-შტადტის აკადემიური სასწავლო ჰოსპიტალი, დერმატოლოგიისა და ალერგოლოგიის დეპარტამენტი, დრეზდენი, გერმანია

ბაზალურ-უჯრედული კარცინომა (ბუკ), უპირა-ტესი ლოკალიზაციით კანის გაშიშვლებულ უბ-ნებზე, ყურის ნიჟარის ჩათვლით, წარმოადგენს ყველაზე ფართოდ გავრცელებულ სიმსივნურ ახალწარმონაქმნს თეთრკანიან მოსახლეობა-ში. უპირატეს მკურნალობად ითვლება ქირურ-გიული ოპერაცია R0-რეზექციის ფარგლებში. მეორე მხრივ, მნიშვნელოვანია ფუნქციური და ესთეტიკური შედეგებიც. ავტორების მიერ წარ-მოდგენილია 79 წლის თეთრკანიანი პაციენტის კლინიკური შემთხვევა ბუკ-ით ყურის ნიჟარის ხვეულის მიდამოში, რომელსაც ჩაუტარდა ერ-თეტაპიანი რეკონსტრუქციული ქირურგიული მკურნალობა Antia-Buch-ის მოდიფიცირებული ნაფლეთის მეთოდით. სტატიაში განხილულია ყურის ნიჟარის ხვეულის მიდამოს ბუკ-ის თა-ვისებურებები და გარეთა ყურის რეკონსტრუქ-ციის ვარიანტები.

PECULIARITIES OF PROLIFERATIVE ACTIVITY OF CERVICAL SQUAMOUS CANCER IN HIV INFECTION

¹Lytvynenko M., ²Shkolnikov V., ³Bocharova T., ³Sychova L., ³Gargin V.

¹Odessa National Medical University; ²National Pirogov Memorial Medical University; ³Kharkiv National Medical University, Ukraine

Mucosal surfaces are the primary sites of most human immunodeficiency virus (HIV) transmission, and thus these tissues are a focus of attention for efforts to prevent HIV infection [20] that is important for future consequences of HIV with neoplastic processes including. Patients with HIV infection have a statistically significant increased risk of developing certain cancer, such as Kaposi's sarcoma, lymphoma, and invasive cervical cancer [8], all of which are considered to be acquired immune deficiency syndrome (AIDS) defining conditions. Cervical cancer is the second most common cancer in women worldwide. It represents one of the most challenging public health problems in developing countries. HIV-infected women have a higher risk of cervical cancer which is an AIDS defining cancer [3,5].

Last decade is characterized by significant search of prognostic signs and especial proliferative peculiarities for carcinoma of different localization [10,16,18,22]. Comparison of different tumours such as breast, thyroid, colon, testis and other [10,17,15,22], the current clinical management of genitals malignancies is lagging behind in terms of utilization of clinically robust molecular tests that can identify patients that are more likely to respond to a given targeted agent, or even those in need of a more aggressive treatment approach based on well-validated molecular prognosticators [12]. One effective means to decrease cervical cancer incidence and death is an early detection of cancer, its precancerous lesions or cervical intraepithelial neoplasia [7] with detection of cellular dysregulation, that could be evidenced clinically by immunohistochemical study of some proteins, such as, p16 and Ki67 [13,23].

Named biomarkers are important for detection, prognosis, and targeted therapeutics of cervical neoplastic transformation and evaluation [2]. Understanding of the molecular events leading to the development and progression of genital malignances, markers of detection, prognostication, and therapy prediction can be exploited in the management of cervical cancer.

The expression of the human Ki-67 protein is strictly associated with cell proliferation. During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0), makes it an excellent marker for determining the so-called growth fraction of a given cell population [19].

In connection with the above, the purpose of our work was detection of proliferative activity in cervical squamous cancer in women with HIV infection with Ki-67 immunohistochemical examination.

Material and methods. There were investigated 24 cases of cervical carcinoma, for which biopsy was performed before the radiation treatment. Materials for the study have been selected with histologically confirmed cervical cancer in 12 patients with HIV (investigated group) and 12 patients without HIV infection (group of comparison). For obtaining relevant results, we included only cases with positive results for human papillomavirus (HPV) infection. HIV infection was determined by a serum enzyme-linked immunosorbent assay (ELISA) with confirmation by Western blot. A CD4 lymphocyte count <100 cells/µL was considered "low." We used the classification of the stages of cervical cancer (FIGO, 2009). Process and extent of tumor (TNM, 2010), under which selected cases treated IIa-IIb (FIGO), T2a-T2b (TNM). Age of women in two groups ranged from 27 to 63 years and averaged 36.5 years.

The material was fixed in 10% neutral buffered formalin and Bouin's fluid for 10-12 hours, were subjected to standard proceeding and embedded in paraffin. From the prepared blocks made serial sections thick 5×10^{-6} m. Slides were stained with hematoxylin and eosin [1], according to van Gieson's. Immunohistochemical examination (IHC) was performed indirect immunoperoxidase reaction [4] with monoclonal antibodies (mAb) to Ki-67, p16; all used mAbs are manufactured by Thermo scientific, USA. The reaction was visualized using a set of UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen (Thermo scientific, USA).

Histological study was performed with microscope «Olympus BX41» followed by morphometric study using "Olympus DP-soft 3.12" program. Staining was scored independently by two observers and a high level of concordance (90%) was achieved. All slides were independently reviewed twice and intra-observer disagreements (<10%) were reviewed a third time followed by a conclusive judgment. Evaluation of expression was performed using a quantitative scale.

Positive Ki-67 expression was diagnosed with nuclear stain in the intermediate and superficial cells. Ki67 staining in basal or parabasal cell was considered as negative. Positive p16 expression was interpreted with a diffuse staining in both nuclear and cytoplasm of basal, parabasal, with or without superficial cells. Unstained, focal or sporadic epithelial staining was considered as negative. Negative control was performed in the same tissue without primary antibodies [7].

Counting of number of investigated structures was performed per 1×10^{-6} m^2 area of the tissue with coincident points x100/number total of points on the grid. All values are expressed as means, standard deviation (SD) and standard error of the mean (SEM) for statistical analysis. Statistical comparison was performed using Mann-Whitney test for statistical analysis. Spearman's rank correlation coefficient (r) was counted for measure of the strength of relationship between paired data [11]. The accepted level of significance was p≤0.05.

The procedure was done strictly in compliance with the Helsinki Declaration after approval from the Regional Ethical Review Board at Odessa National Medical University, protocol 3, 17th October 2011.

Results and their discussion. As we wrote above age of women in two groups ranged from 27 to 63 years and averaged 36.5 years, but separation of women age in group with/without HIV realized in significant differences. So, averaged age was 32.7 years in group with HIV infection, and 38.2 years in group without HIV infection.

The 24 cases were histopathological diagnosed as follows: well-differentiated keratinizing squamous cell carcinoma (7 cases), moderately differentiated nonkeratinizing squamous cell carcinoma (13 cases) and poor differentiated nonkeratinizing squamous cell carcinoma (4 cases). The distribution of histological variants of cervical cancer by the studied groups is presented in the Table 1.

As a result of IHC it was detected that positive Ki-67 expression and positive p16 expression have been revealed in 100% and 95.2% cases respectively, but percentage of cell with positive staining was uneven in investigated groups. So, percentage of cell with positive staining Ki67 was ranged from 11.32 to 85.4 % (averaging 48.8%) in group without HIV. But it was ranged from 27.41 to 93.4 (averaging 62.5%) in HIV group (Table 2). It should be noted that dysplastic cells have been revealed with positively responding nuclei to Ki-67 in all cases, mostly outside, layers of the epithelium, the intensity of

Table 1. Distribution of histological variants of cervical cancer

Histological types	Cervical cancer without HIV	Cervical cancer with HIV
well-differentiated squamous cell carcinoma	5	2
moderately differentiated squamous cell carcinoma	6	7
poor differentiated squamous cell carcinoma	1	3

Table 2. Evaluation of expression for immunohistochemical staining (% of positive cells)

Histological types	Ki-67		p16	
	No HIV (n=12)	HIV (n=12)	No HIV (n=12)	HIV (n=12)
Well-differentiated squamous cell carcinoma	21.3±2.4	34.7±3.8*	49.2±4.7	64.7±4.3*
Moderately differentiated squamous cell carcinoma	45.8±4.2	63.7±4.3*	69.7±5.8	68.9±6.1
Poor differentiated squamous cell carcinoma	79.4±3.7	89.2±5.1*	68.4±5.8	61.7±7.3
Averaging level	48.8±5.2	62.5±5.6*	62.4±4.3	65.1±8.4

* - p<0.05 significant between groups with and without HIV

the reaction was moderate and high in peritumoral tissue. In some cases of group without HIV, the cells of the basal layer were Ki-67-negative. At the same time, as we move into the tumor, cells with positive nuclear reaction for Ki-67 detected as primarily high intensity in all layers of the cervical epithelial layer. Almost all cell nuclei were Ki-67-positive reactions with high intensity in areas that are suspicious as microinvasion in both groups.

IHC localization and intensity of response to Ki-67 have been depending of invasive growth varies on the degree of differentiation. Thus, in poor differentiated squamous cell carcinoma positive nuclear reaction for Ki-67 was detected in the majority of cells without a particular pattern, whereas in the stratum cancer, in the central parts Ki-67 - positive cells interspersed with Ki-67 - negative. But comparison of same level differentiation case resulted in more active proliferative process in all subgroups - well-, moderately- and poor differentiated squamous cell carcinoma.

As we wrote above p16 expression has been revealed in 95.2% cases, due to presence of 3 cases from HIV group with unclear pale nuclear and cytoplasmic staining, that we recognize as negative expression. Percentage of cell with positive p16 staining was uneven in investigated groups as and Ki-67 expression (Table 2).

The p16-positive immunostain was diffuse and strong at nuclear and cytoplasmic localization, without differences regarding the intensity of reaction in different level of differentiated squamous cell carcinoma degree for first point of view. Simultaneously we observed weak intensity stain in the cytoplasm and nucleus of non-epithelial cells, such as fibroblasts, inflammatory cells, vascular endothelial cells. P16 immunostaining was present in basal and parabasal cells mainly from the lower third of the epithelium till two thirds epithelium in peritumoral tissue. The p16 reaction was observed also in few epithelial cells from the upper layers with no intensive staining.

Women with HIV infection have an increased risk of developing certain malignancies. These malignancies are commonly human papillomavirus (HPV)-related reflecting the high rate of coinfection with HPV m women with underlying HIV infection. These women also have a high incidence of premalignant HPV-related changes, such as high-grade squamous intraepithelial lesions as diagnosed on Pap smears and cervical intraepithelial neoplasia on cervical biopsy. Screening recommendations for HIV-infected women reflect the need for vigilance in detecting and treating these lesions early. In addition, recent interest has focused on the use of cervical cancer screening, employing HPV-testing techniques, and on HPV vaccination in younger women to prevent initial infection and the subsequent development of cervical and other HPV-related cancers [8]. Risk factors for cervical cancer include sexual intercourse at an early age, multiple sexual partners, tobacco smoking, long-term oral contraceptive use, low socioeconomic status, immunosuppressive therapy, and micronutrient deficiency. Persistent infection by oncogenic, high-risk strains of HPV is strongly associated with the development of cervical cancer [9] and as all patients with HIV were observed with positive HPV so we select patients with same indicators by HPV for group of comparison. Distribution of histological variants of cervical cancer by the studied groups is approximately same with insignificant tendency to less differentiate for group with HIV.

Proliferation of appeared malignant tumor is important characteristic for prognosis. Ki-67 is a nuclear protein associated with cell proliferation and ribosomal RNA transcription. It is found in all active phases of the cell cycle and increasing the fraction Ki-67 positive tumoral cells is associated with a worsening of the prognosis for course of tumor [19]. Status of Ki-67 could be detected as an independent predictor disease free survival and presence of numerous Ki-67 positive stained cells is expected results for developed cervical carcinoma in our slides.

Level of Ki-67 is progressively increased in both investigated groups, but level of proliferation is significantly higher in group with HIV. So, averaging level for all histological types in group without HIV was 48.8±5.2% with 62.5±5.6% in group with HIV. Level of proliferation was more pronounced in group with HIV and in all histological types of cervical cancer.

The rates of p16 and Ki67 expressions were directly associated with the severity of cervical lesions but should be interpreted result with caution [7]. Therefore, p16 overexpression, identified by immunostaining or enzyme-linked immunosorbent assay, can be considered as a marker of HPV infection and of activated expression of viral oncogenes and virus-induced cell cycle deregulation [14, 21]. So, we observed significant difference between histological subgroups for Ki67, but only one subgroup

(well-differentiated squamous cell carcinoma) with significant difference for p16: 49.2±4.7% for non HIV and 64.7±4.3% for HIV (p < 0.05). For moderately and poor differentiated squamous cell carcinoma difference of p16 expression was statistically unreliable. Such result must be evaluated carefully as there are data that meta-analysis of p16 overexpression could be associated with a favourable prognosis in patients with cervical cancer. Assessment of p16 expression could provide better prognostic information for patients with cervical cancer. Large scale, multicentre and well-matched cohort studies are warranted to clarify the prognostic effect of p16 expression on the outcome of cervical cancer [6]. Simultaneously such hesitation of p16 could be result of influence HPV infection in HIV group.

Conclusion. Proliferative activity in cervical squamous cancer in women with HIV infection is characterized higher level of Ki-67 with averaging level for all histological types of squamous cell carcinoma 62.5±5.6% that one and half more than in group without HIV. Depend of histological type, expression of Ki-67 increased from 4.7±3.8% in well-differentiated squamous cell carcinoma till 89.2±5.1% in poor differentiated squamous cell carcinoma for group with HIV (21.3±2.4% till 79.4±3.7 in group without HIV accordingly).

This work should be regarded as a preliminary investigation, with promising results, which provides a strong motivation for increasing the research efforts in the quantitative analysis of the invasive squamous cell carcinoma in women with HIV.

Conflict of Interest Statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUMMARY

PECULIARITIES OF PROLIFERATIVE ACTIVITY OF CERVICAL SQUAMOUS CANCER IN HIV INFECTION

[1]Lytvynenko M., [2]Shkolnikov V., [3]Bocharova T., [3]Sychova L., [3]Gargin V.

[1]Odessa National Medical University;[2]National Pirogov Memorial Medical University; [3]Kharkiv National Medical University, Ukraine

Patients with human immunodeficiency virus (HIV) infection have a statistically significant increased risk of developing cervical cancer. The expression of the human Ki-67 protein is strictly associated with cell proliferation. The purpose of our work was detection of proliferative activity in cervical squamous cancer in women with HIV infection.

We investigated 24 cases (12 patients with HIV and 12 patients without HIV infection) of cervical carcinoma, where biopsy had been performed before the treatment. According to histopathological diagnoses, moderately and poorly differentiated squamous cell carcinoma (7, 13 and 4 cases respectively) was determined. Mean age of women in the group with HIV infection was 32.7 years, and 38.2 years in the group without HIV infection. Detection of protein Ki-67 expression was performed with nuclear staining in the intermediate and superficial cells.

The results of this work show that proliferative activity of cervical squamous cancer in women with HIV infection is characterized by a higher level of Ki-67 with averaging level for all histological types of squamous cell carcinoma 62.5±5.6% that is one and half times higher than in group without HIV infection. Depending on a histological type, expression of Ki-67 has increased from 4.7±3.8% in well-differentiated squamous cell carcinoma up to 89.2±5.1% in poorly differentiated squamous cell carcinoma for group with HIV, and from 21.3±2.4% to 79.4±3.7 in group without HIV.

РЕЗЮМЕ

ОСОБЕННОСТИ ПРОЛИФЕРАТИВНОЙ АКТИВНОСТИ ПЛОСКОКЛЕТОЧНОГО РАКА ШЕЙКИ МАТКИ ПРИ ВИЧ-ИНФЕКЦИИ

[1]Литвиненко М.В., [2]Школьников В.С., [3]Бочарова Л.В., [3]Сычева Л.В., [3]Гаргин В.В.

[1]Одесский национальный медицинский университет; [2]Винницкий национальный медицинский университет им. М.И. Пирогова; [3]Харьковский национальный медицинский университет, Украина

Пациентки, инфицированные вирусом иммунодефицита человека (ВИЧ), имеют статистически значимый повышенный риск развития рака шейки матки. Экспрессия белка Ki-67 человека строго связана с пролиферацией клеток.

Целью исследования явилось определение пролиферативной активности у женщин с ВИЧ-инфекцией при цервикальном плоскоклеточном раке.

Наблюдались 24 женщины с карциномой шейки матки (12 - с ВИЧ и 12 - без ВИЧ-инфекции), у которых по результатам биопсии, взятой до лечения, гистопатологический диагноз соответствовал высоко, умеренно или низко дифференцированному плоскоклеточному раку (7, 13 и 4 случаев, соответственно). Средний возраст женщин составил 32±0,7 года в группе с ВИЧ-инфекцией и 38±0,2 года в группе без ВИЧ-инфекции.

Проведено иммуногистохимическое и гистологическое исследование полученного материала, определено значение показателей Ki-67.

Полученные данные обработаны с применением теста Манн-Уитни для статистического анализа, также высчитывали ранговый коэффициент корреляции Спирмена (r). Уровень достоверности соответствовал p≤0,05.

Результаты проведенного исследования выявили пролиферативную активность при цервикальном плоскоклеточном раке у женщин с ВИЧ-инфекцией с более высоким уровнем Ki-67, средний уровень для всех гистологических типов плоскоклеточной карциномы составил 62,5±5,6%, что в полтора раза выше, чем в группе без ВИЧ. В зависимости от гистологического типа экспрессия Ki-67 увеличилась с 4,7±3,8% при высоко дифференцированной плоскоклеточной карциноме до 89,2±5,1% при низко дифференцированной плоскоклеточной карциноме для группы с ВИЧ, а в группе без ВИЧ эти показатели варьировали в пределах от 21,3±2,4% до 79,4±3,7 соответственно.

Авторы указывают 2 типа дифференциации опухоли, но разбивают на 3 раздельные количества пациентов, и пишут "соответственно". Пожалуйста, уточните: необходимо или указать третий соответствующий тип дифференциации опухоли, или же сократить соответствующие количества до 2-х

რეზიუმე

საშვილოსნოს ყელის ბრტყელუჯრედოვანი
კიბოს პროლიფერაციული აქტივობის თავისე-
ბურებანი აივ-ინფექციის დროს

¹შ. ლიტვინენკო, ²ვ. შკოლნიკოვი, ³ლ. ბოჩაროვა,
³ლ. სიჩოვა, ³ვ. გარგინი

¹ოდესის ნაციონალური სამედიცინო უნივერსიტე-
ტი; ²ვინიცის მ. პიროგოვის სახელობის ნაციონა-
ლური სამედიცინო უნივერსიტეტი; ³ხარკოვის ნა-
ციონალური სამედიცინო უნივერსიტეტი; უკრაინა

აივ-ინფიცირებულ პაციენტ ქალებს აქვთ საშვი-
ლოსნოს ყელის კიბოს განვითარების სტატის-
ტიკურად სარწმუნოდ მომატებული რისკი. ცილა
Ki-67-ის ექსპრესია მკაცრადაა დაკავშირებული
უჯრედების პროლიფერაციასთან.

კვლევის მიზანს შეადგენდა პროლიფერა-
ციული აქტივობის განსაზღვრა აივ-ინფექციის
მქონე ქალებში ცერვიკული ბრტყელუჯრედოვანი
კიბოს დროს.

გამოკვლეულია 24 ქალი საშვილოსნოს
ყელის კარცინომით (12 – აივ-ინფექციით, 12 –
აივ-ინფექციის გარეშე),რომელთა ჰისტოპათოლო-
გიური დიაგნოზი, მკურნალობამდე აღებული
ბიოფსიის შედეგების მიხედვით, შეესაბამებოდა

მადალ-, საშუალო- ან დაბალდიფერენცირებულ
ბრტყელუჯრედოვანი კიბოს (7, 13 და 4 შემთხ-
ვევა, შესაბამისად). აივ-ინფექცირებულ ქალთა
საშუალო ასაკი იყო 32±0,7 წელი, აივ-ინფექციის
გარეშე - 38±0,2 წელი. მიღებული მასალა შეს-
წავლილია იმუნოჰისტოქიმიურად და ჰისტოლო-
გიურად, განსაზღვრულია Ki-67-ის მაჩვენებლები.
მიღებული მონაცემები სტატისტიკურად და-
მუშავებულია მან-უიტნის ტესტით,ასევე,გამოით-
ვლებოდა სპირმენის კორელაციის რანჟირებული
კოეფიციენტი (r). სარწმუნობის მაჩვენებელი
შეადგენდა p≤0,05.

ჩატარებული კვლევის შედეგების საფუძ-
ველზე აივ-ინფექციის მქონე ქალებში ცერვიკული
ბრტყელუჯრედოვანი კიბოს დროს პროლიფერა-
ციული აქტივობა გამოვლინდა Ki-67-ის უფრო
მადალი მაჩვენებლების შემთხვევაში; საშუალო
მაჩვენებელი ბრტყელუჯრედოვანი კიბოს ყვე-
ლა ჰისტოლოგიური ტიპის დროს შეადგენდა
62,5±5,6%-ს, რაც ერთნახევარჯერ მეტია, ვიდრე
ჯგუფში აივ-ინფექციის გარეშე. აივ-ინფიცირე-
ბულთა ჯგუფში Ki-67-ის ექსპრესია ჰისტოლო-
გიური ტიპის მიხედვით გაიზარდა 4,7±3,8%-დან
(მადალდიფერენცირებული ბრტყელუჯრედოვანი
კიბოს დროს) 89,2±5,1%-მდე (დაბალდიფერენცი-
რებულ ბრტყელუჯრედოვანი კიბოს დროს). აივ-
ინფექციის გარეშე კი ეს მაჩვენებლები ვარირებდა,
21,3±2,4% -დან 79,4±3,7-მდე, შესაბამისად.

IMMUNOHISTOCHEMICAL ANALYSIS OF APPENDIX CELL WALL INFILTRATE IN ACUTE PHLEGMONOUS APPENDICITIS

¹Kuyun L., ²Kurchenko I.

¹Bogomolets National Medical University, Kyiv; ²Bukovinian State Medical University, Chernivtsi, Ukraine

Appendicitis is a common pathological condition in surgery found in 8.9 [2] to 10 [4] patients per 10,000 people. It is associated with acute systematic and local inflammation [1,8].

Morphological examination of the appendix infiltrate in 200 patients with acute phlegmonous appendicitis has shown that lymphocytes prevail in the subserous membrane [3].

Cross-section examination of 71 patients has shown that increased active B-lymphocytes (HLA-DR+CD19) and T-lymphocytes (α/β TCR and CD3/RA) content is a clear indicator of acute inflammation during appendicitis [5]. Another research has shown natural killer cells content in peripheral blood to be statistically lower in patients with perforation compared to those found in patients who did not have post-surgical exacerbation [6].

Other sources lack data regarding immunohistochemical properties of the proliferative mucous infiltrate of the appendix during acute phlegmonous appendicitis, where the levels of expression of CD3, CD4, CD8, CD20, CD45, CD45R0, CD68, and Ki-67 positive cells are defined.

Objective: analyze the content of the mucous infiltrate of cells of the lamina propria of the appendix using immunohistochemical analysis in acute phlegmonous appendicitis patients.

Materials and methods. The research included 21 patients with acute phlegmonous appendicitis. The control group included 15 patients who did not exhibit the inflammation of the intraperitoneal cavity.

Histological samples were obtained from 21 patients in Kyiv municipal hospital #8. Appendix tissue was