CARCINOMA OF THE CERVIX AT MUHIMBILI NATIONAL HOSPITAL, TANZANIA: Diagnosis and Therapeutic Implication of the Small Cell Type

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine (Anatomical Pathology) of the University of Dar es salaam

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Carcinoma of the cervix at Muhimbili National Hospital; Tanzania: Diagnosis and therapeutic implications of the small cell type,* in partial fulfillment of the requirements for the degree of Master of Medicine (Anatomical Pathology)

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DECLARATION

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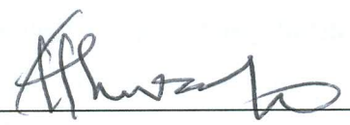
I, Dr. Henry Adamson Mwakyoma, declare that this dissertation is my own original work and that has not been presented to any other University for a similar or any other degree award.

Signature  .

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DEDICATED

TO

MY WIFE LEAH,

MY DAUGHTER ELINA,

AND SONS

REUBEN

AND

ADAM

WITH LOVE

ABSTRACT

Carcinoma of the cervix is the most common malignancy worldwide after breast cancer, majority occurring in developing countries. One histological type of cervical cancer, known as small cell carcinoma (SMCC) is relatively rare but more aggressive when it occurs, posing problems in management. It is established that human papillomavirus (HPV) is the etiological agent for squamous intraepithelial lesions (SIL) and cervical cancer. Recently there have been suggestions that cervical cancer occurs more often in HIV infected women. Immunosuppression, caused by human immunodeficiency virus (HIV) infection, which in the terminal stages causes acquired immunodeficiency syndrome (AIDS), leads to persistence of HPV and therefore increased risk of SIL and cancer. Information on the association of HIV infection and cervical cancer is rare in Tanzania. Likewise the frequency of small cell carcinoma, and whether it has any association with HIV infection is lacking. In order to plan the management of cervical cancer and particularly SMCC, it is important to know its magnitude and also interaction with HIV infection. For this ressoan a prospective study was carried out at Muhimbili National Hospital.

Results show that 86.2% clinically diagnosed patients were histologically confirmed as cervical cancer while 13.8% were non-cancerous lesions. Mean age of cervical cancer was 49.6 years, similar to what has been reported before. A geographical variation in the distribution of cervical cancer in Tanzania was noted which may be a result of different socio-cultural factors in the different zones. Sexual related risk factors for cervical cancer were identified as mean age at first exposure to sexual intercourse, marriage and

pregnancy below 19 years. Polygamy and unstable marital status were noted as risk factors although there was no statistically significant difference ($p<0.70$). The number of pregnancies as a risk factor for cervical cancer were only significant when they were seven and above ($p<0.01$).

Majority (90%) of patients were diagnosed when the disease was in late stages, an indication of poor prognosis. Histologically, squamous cell carcinoma (SCC) was the most common (86.5%). SMCC accounted for 10.9% of all cervical cancer and neuroendocrine carcinoma (NE) for 38.1% of all SMCC.

Out of 42 patients with cervical cancer, 61.9% had demonstrable HPV and out of these 10 (23.8%) and 16 (38.1%) were small cell and non-small cell carcinomas respectively. The mean age and clinical stage of small cell carcinoma were slightly higher than that of non small cell carcinoma although there was no statistically significant difference irrespective of HPV serostatus ($p<0.90$).

Severe immunodeficiency caused by HIV was associated with advanced clinical stages of cervical cancer ($p<0.002$), but non of the SMCC was associated with HIV infection. It is concluded that cervix cancer is a major problem in women especially those in active age groups. Sexually related factors besides HPV are risk factors involved in the pathogenesis of the disease. Preventive measures directed against these risk factors and early diagnosis of cervix cancer are recommended in order to reduce to a minimum the incidence and burden of the disease at national level.

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ABBREVIATIONS

ABC	- Avidin biotin complex
AIDS	- Acquired immonodeficiency syndrome
CD	- Cluster of differentiation
CIN	- Cervical intraepithelial neoplasia
DAB	- Diamino benzidine
EMA	- Epithelial membrane antigen
FIGO	-International federation of gynaecologists and Obstetricians
H & E	- Haematoxylin and eosin
HD	- Hodgkin's lymphoma
HIV	- Human immuodeficiency virus
HPV	- Human papillomavirus
Mab	- Monoclonal antibody
MNH	- Muhimbili National Hospital
N-CAM	- Neural cell adhesion molecule
NHL	- Non – hodgkin's lymphoma
NOSCC	- Non small cell carcinoma
NSCC	- Non – specific chronic cervicitis
NSE	- Neurone specific enolase
P	- p value
SIL	- Squamous intraepithelial lesions
SMCC	- Small cell carcinoma

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TBS	- Tris buffered saline
WB	- Western blot
WHO	- World Health Organization
X^2	- Chi-square
>	- Greater than
<	- Less than
≥	- Greater than or equal to

INTRODUCTION

Cervical cancer is the most common malignancy in women worldwide after breast cancer. It is estimated that about 500,000 new cases of cervical cancer are diagnosed every year, accounting for 15% of all cancers in women[1,2]. Studies around the world have implicated human papillomavirus (HPV) as the primary sexually transmitted agent in the development of SIL[3]. The critical role of HPV in the causation of cervical cancer was recently confirmed by Ho et al., who reported that persistent detection of HPV DNA, and high viral load were associated with persistent cervical dysplasia[4]. Invasive cervical cancer is therefore the end result of a continuum of progressive, consecutive stages which begins with precursors variably called cervical dysplasia, cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL)[5]. Although most follow-up studies have shown spontaneous regression of a large proportion of both low – grade and high-grade SIL among human immuodeficiency virus (HIV) seronegative women[6], a substantial proportion of dysplastic lesions have been shown to progress to a more advanced stage in women infected with HIV[7]. HIV infection, by virtue of its depression of the immune system, predisposes to malignancies, the most common being Kaposi's sarcoma and Non-Hodgkin's lymphoma (NHL)[8]. Several other tumours, such as Hodgkin's disease (HD)[9], alimentary tract tumours[10] and anorectal cancers[10] have been reported in association with HIV. Other studies have indicated increased risk of HPV and invasive cervical cancer in HIV infected women [11,12,13], leading to inclusion of cervical cancer as an AIDS defining illness in women[14]. Impaired cellular and local immunity have been thought to contribute to the increased

risk of persistent HPV and SIL among HIV infected women[15]. Since the first report by Wentz and Regan, it has been recognized that cervical small cell carcinomas representing 20% to 30% of cervical cancers, behave more aggressively, metastasize early and recur more frequently than other variants[16]. In patients with HIV infection, cervical cancer follows a more aggressive course[17]. It has also been shown that in patients with small cell carcinoma, the course of the disease is even more aggressive than other types of cervical carcinoma.

LITERATURE REVIEW

1. Small cell carcinoma

Small cell carcinoma of the cervix is an uncommon variant of cervix cancer with particularly poor prognosis[18]. This tumour represents 2 – 5% of all cervical carcinomas and 20 –35% of all malignant neoplasms. It is usually seen in younger women presenting with more aggressive clinical course than that of usual squamous cell carcinoma of the cervix[20,21]. Polypeptide hormone production and paraendocrine syndromes have been described in association with these tumours[21] and presents as highly aggressive and most often poorly differentiated tumour[27]. Patients present with a variable degree of tumour differentiation sometimes secreting detectable levels of neuron specific enolase (NSE), prolactin, glucagon[22] inappropriate antiduretic hormone (IADH)[22] and serum adrenocorticotropic hormone (ACTH)[23].

2. Association of small cell carcinoma with HPV and HIV infections

(a) HPV infection

Human papillomavirus (HPV) type 18 is found in most small cell carcinoma and likewise HPV 16 has also been reported[21]. Infection with HPV is thought to play an important role in the development of epithelial neoplasia in patients with HIV infection. HIV tat protein is known to up-regulate the expression of HPV in tissue, a finding that would be expected to lead to increased frequency of HPV–induced lesions[24]. Furthermore, E6 oncoprotein of HPV16 interacts with p53 tumour suppressor gene product, causing its inactivation, which may promote the development of cervical cancer[25]. It can therefore be assumed that HPV- associated neoplasia including small cell cervical cancer will be more frequent in HIV infected patients.

(b) HIV infection.

The incidence of epithelial neoplasms in patients infected with HIV has markedly increased, commonly occurring in oral, cervical and anorectal locations. Women with AIDS have been found to have two-fold increased risk of developing cervical cancer compared with the general population[26]. HIV-infected women have been shown to have dysplasia rates five to ten times higher than non-HIV infected women[27]. Furthermore, risk of developing cervical disease in association with HPV is clearly higher in the setting of HIV infection and there are suggestions that this risk increases as the degree of HIV- associated

immunosuppression increases[28]. The association of HIV and small cell cervical cancer has not been conclusively studied and there are no reports from Tanzania.

(c) HPV, HIV, CD^{4+} lymphocyte count and Cervix cancer.

A number of studies have shown the association of HPV, HIV and cervical cancer using either HIV positivity or degree of severity of HIV – associated disease as a surrogate marker for the level of immune status. Ho et al.,[4] studied the association of HIV, HPV and CD^{4+} cell count in women with cervical cancer and found that among 207 primary intravenous drug using women, young age (less than 35 years) and HIV positivity were the only independent covarietes associated with HPV DNA positivity. The prevalence of HPV increased with decreasing CD4+ count, from 23% among immunocopetent HIV negative subjects to 45% in mild or moderate immunosuppression (HIV positive and CD4 cell count more than 20%) to 61% in severe immunosuppression (CD4 cell count less than 20%). In another study[29], the association of squamous intraepithelial lesions (SIL) and T-lymphocytes subset were investigated and found that the risk of SIL increased with decreasing CD4+ lymphocyte count (P-value 0.02), although this was statistically significant for only those with CD4 cell count less than 200 cells/mm^3. The risk of SIL decreased significantly by 40% and 21% for each increase of 10% of $CD4^+$ and $CD8^+$ T cells, respectively.

3. **Diagnosis of small cell carcinoma of cervix**

Light microscopic observation of Grimelius and immunohistochemical stained sections and electron microscopic observation can be performed in addition to H&E on cases suspected of having small cell carcinoma of cervix.

(i) Grimelius stain:

This is a useful histochemical stain for argyrophil neuroendocrine granules, carcinoid tumours and A-cells of pancreas islets which stains brown/black to indicate the presence of argyrophil neuroendocrine material in the tumour[21].

(ii) Immunohistochemical stains

Immunohistochemical markers for neuroendocrine differentiation include anti-CD57, Chromogranin A (CgA), Synaptophysin and Neuron specific enolase (NSE) which was employed in this study. These area used to confirm neuroendocrine differentiation.

4. **Management and prognosis of small cell carcinoma**

Since small cell neuroendocrine tumours behave more aggressively, metastasize early and recur more frequently than other variants, they must therefore be promptly diagnosed and managed[20]. Current data suggest that at least 50% of stage 1b lesions will be found to have nodal spread, metastases to the lungs, bones, liver, brain and supraclavicular lymphnodes[20]. Recurrences are seen early with survival rate of less than 20%[20]. Current management suggests that aggressive surgery and irradiation should be supplemented with chemotherapy for better results. Treatment results correlate strongly with the stage of disease. In early stage carcinoma of the cervix in

general, there is no convincing evidence that the result with irradiation or surgery are statistically different. Cure rates are approximately 90% in stage I and 60% in stage II and about 33% in stage III disease[20]. It is recommended that multimodal therapy, combining radical surgery and radiation with cytotoxic chemotherapy may provide patients with small cell carcinoma of the cervix the best chance of cure[20]. It is therefore important to diagnose correctly and differentiate neuroendocrine carcinomas of the cervix from other carcinomas of this location, so as to plan effective curative management.

STATEMENT OF PROBLEM

HIV infection and cervical cancer are major public health problems among women in Tanzania where about 11,673 people were estimated to have been infected with HIV from January to December 2000 in the 20 regions of Tanzania Mainland[30]. Of these, 5,719 (49%) were females with a peak age between 20 – 49 years. Although the prevalence of cervical cancer in the general population is unknown, hospital based studies show that cervical cancer accounts for approximately 40% of all cancers diagnosed in women[31] and about 30% of all gynaecological admissions[32]. Local studies have shown major regional variations in the number of reported cases of cervical cancer in Tanzania[33]. In general, the information on the prevalence of small cell carcinoma of the cervix is limited and there are no reported studies on small cell carcinoma of the cervix in both HIV seropositive and seronegative women in Tanzania. Small cell carcinoma is thought to be a distinctive entity with a poor prognosis and within this entity the most aggressive tumours are those with neuroendocrine

differentiation[34]. Currently the diagnosis of small cell carcinoma at the department of Pathology Muhimbili National Hospital is mainly based on haematoxylin and eosin stained sections, but there has been a trend to refer to this tumour as neuroendocrine carcinoma, which require demonstration of cytoplasmic granules[18]. Sheridan et al[18], have shown that not all patients diagnosed as having small cell carcinoma of the cervix by light microscopy, showed evidence of neuroendocrine differentiation. Therefore it is proposed that the present criteria for diagnosing small cell carcinoma is inadequate. Instead diagnosis should rely not only on light microscopy of haemotoxylin and eosin (H&E) stained sections, but also on histochemical stains such as Grimelius for the demonstration of neuroendocrine differentiation, immunohistochemistry using antibodies for the detection of neurosecretory granules in small cell carcinoma and finally by considering the distinctive clinical behaviour of the tumour. Patients with small cell carcinoma, especially with neuroendocrine differentiation and who are HIV positive, require aggressive management since in these patients cervical cancer follows a more severe and short course[19]. Relative to other histological types, small cell carcinoma of the cervix is an aggressive neoplasm with a high rate of lymphovascular space involvement (LVSI) and lymphnode metastasis (LNM) despite smaller depth of invasion (DI) and tumour size[20]. It was concluded from this data that a multimodality therapy combining radical surgery, radiation and cytotoxic chemotherapy may provide the best chance for cure to the patients[20]. However, at Muhimbili National Hospital there is no distinction on treatment between small cell carcinoma and non-small cell

carcinoma. The correct histological diagnosis of small cell carcinoma will help to plan appropriate management.

HYPOTHESIS:-

1. Small cell carcinoma of the cervix is not uncommon in Tanzania
2. Lack of correct histological diagnosis of small cell carcinomas of the cervix may lead to improper management.

OBJECTIVES:

1. BROAD OBJECTIVE:

To study the proportion, distribution and pathogenic factors related to small cell carcinoma of the cervix among women with cervix carcinoma.

2. SPECIFIC OBECTIVES

(i) To determine the aetiologic risk factors of cervical cancer.

(ii) To determine the age distribution of women with different types of cervical carcinomas.

(iii) To determine the proportion of small cell carcinomas of the uterine cervix by histology among cervical carcinomas.

(iv) To classify small cell carcinomas of the cervix into subtypes.

(v) To compare HIV serostatus in patients with small cell carcinomas and with non-small cell carcinomas of the cervix.

(vi) To compare the clinical stage for small cell carcinomas and non-small cell carcinomas of the cervix in both HIV seropositive and seronegative patients.

(vii) To compare the specificity and sensitivity of NSE and Grimelius stains on staining characteristics of neuroendocrine granules in small cell carcinoma.

(viii) To compare the proliferative-index of positive Ki-67 tumour cells between small cell and non small cell carcinomas.

MATERIALS AND METHODS

Study population

This study was a prospective one and included all new patients admitted in gynaecological wards at Muhimbili National Hospital (MNH) with suspected cervical cancer for a period of one year from 1st February 2001 to 1st February 2002. However, some patients who were admitted during weekends were not included in the study due to logistic reasons.

Sample size

Included 224 patients whose biopsies were studied prospectively.

Specimens

Punch biopsy specimens were taken from patients by a specialist in gynaecology or experienced senior resident and during this time they also did clinical staging of patients using the FIGO classification the. The biopsies were then put immediately in a bottle containing 10% neutral buffered formalin. After overnight fixation the tissue were preserved in 70% alcohol until processed. Blood was taken from each patient for CD4 and CD8 cells assay by flow cytometry and serum for HIV ELISA and Western Blot.

METHODS:

Elisa / Western Blot and flow cytometry:

Blood samples were collected in empty sterile vacutainer tubes and allowed to clot after which sera was removed by a pipette and put in cryotubes and stored at – 20°c until testing time. The assay was done by ELISA (Behring, Murburg Germany) which is a sandwich assay made up of recombinant proteins, and Wellcozyme HIV–1 recombinant ELISA (Murex Danford, U.K.) which is a competitive assay. The test procedure followed the manufacturer's instructions[35].Washing of ELISA plates was done manually. A confirmatory Western Blot (WB) analysis (HIV blot 2.2. Diagnostic Biotechnology (Pte) science Singapore) was done. WB results were interpreted according to WHO criteria requiring reactivity to at least two envelope proteins for positivity (WHO 1995)[35].

The FACS count system (Beckon Dickson Immunocytometry system, San Jose CA, USA) was used for determination of lymphocyte subsets. Blood samples rejected by the FACS count system was analysed using the FACScan analyzer (Beckon Dickson immunocytometry system San Jose CA, USA). Other haematological indices were measured using standard methods.

Haematoxylin and Eosin (H&E)

The tissue specimens were embedded in paraffin wax (melting point 56°c) and processed in the usual manner. Five micrometer sections were cut processed and eventually stained routinely using H&E on microscopic glass slide for morphological diagnosis.

Histochemistry

Grimelius stain and NSE were performed on cervical small cell carcinomas for the detection of neuroendocrine differentiations according to already established procedures in the laboratory. Cells giving positive results stained brown / black colour in the cytoplasm with light brown nuclei. The background gave yellow or green shades.

Immunohistochemistry:-

Antibodies were applied against various antigens (Table 1) which were tested on paraffin sections after antigen retrieval following manufacturer's instructions. Briefly, the following steps were followed:

Five microns (5μ) thick paraffin sections were mounted on pre-treated slides and heated in an oven for 30 minutes. Deparaffinization of sections using 4 changes in xylene for 5 minutes each was done followed by re-hydration in graded changes of alcohol to water. The sections were then rinsed in 2 changes of distilled water of 5 minutes each.

This was followed by citrate antigen retrieval (citrate buffer pH 6.0) in a microwave and allowed to cool to room temperature for 30 minutes. The section were then washed in Tris Buffered Saline (TBS) pH 7.6.

Blocking of endogenous peroxidase activity was then done using 30% Hydrogen peroxide (30% H_2O_2) in methanol for 25 minutes and followed by TBS wash (pH 7.6) for 5 minutes, 3 times. Cross reacting antigens were blocked by incubating sections in 1% normal horse serum (1% NHS) 1:20 at room temperature for 20 minutes. Excess NHS was tapped off and slides placed in leveled incubation chambers.

The primary antibody was then applied covering the whole section and incubated overnight at 4°C. Next morning the primary antibody was washed off using TBS (pH 7.6), followed by incubation with biotinylated secondary antibody (biotinylated horse anti-mouse) 1:200 for 30 minutes at room temperature in a humid chamber.

After washing, the sections were incubated with Vectastain ABC reagent diluted at 1:20 according to the recommendation of the manufacturer for 30 minutes at room temperature.

Sections were then developed in 0.01% DAB and 0.24% H_2 0_2 in TBS under microscopic guidance after which they were washed in water. Counterstaining was done with Harris haematoxylin and "bluing" of sections in running water.

Dehydration of sections was done in graded alcohol to xylene and cover slips mounting with DPX. Evaluation of immunostains was done in a Carl Zeiss Axiomatic microscope.

Statistical analysis

The difference between cases and controls were tested for significance using Chi-square test (X^2) of various continuous variables which included gravidity, parity, clinical stages of cervix cancer and HIV serostatus. Sensitivity and specificity was tested on reactivity of neuroendocrine cells in small cell carcinoma between Grimelius stain and neuron specific enolase and (NSE) using statistical software (Analyse-it software Ltd).[36] Statistically significant differences were considered those with $p< 0.05$.

Questionnaire

During blood collection, patients clinical data including age, marital status, place of residence in the past 5 years, number of pregnancies, number of children number of wives to one husband and sexual – related risk factors were also asked.

(see appendix A)

Criteria for controls

Controls were patients who were admitted in the same gynaecological wards at MNH during the period of study but lacked the factor which was under investigation on cases. The following criteria was used in the selection of controls.

(i) The gravidity parity and number of wives for one husband were age matched women without cervical cancer and followed by stratified sampling method.

(ii) For HIV serostatus, women were aged matched patients with cervical cancer but HIV negative followed by stratified sampling method.

(iii) For clinical staging of cervical cancer, they were age matched with cervical cancer but HIV negative followed by stratified sampling method.

(iv) Controls between small cell and non-small cell carcinoma of the cervix were age matched and then stratified sampling method (Table 5).was done.

(v) The controls obtained on the distribution of clinical staging of cervix cancer among HIV positive cases (Table 8) had cervix cancer, were HIV seronegative, age matched and than stratified sampling method was performed.

Definition of small cell carcinoma

The term small cell carcinoma of the cervix in this study imply may imply one of the following:-

(i) Carcinoid tumour

(ii) Squamous cell carcinoma, small cell non-keratinizing type

(iii) Poorly differentiated non-keratinizing carcinoma

(iv) Small "oat" cell carcinoma- poorly differentiated or undifferentiated carcinoma.

(v) Neuroendocrine carcinoma

The histologic criteria for the diagnosis of small cell carcinoma included the tumour which showed sheets of small - sized cells, round to oval , with scanty cytoplasm.and had hyperchromatic nuclei with finely stippled chromatin and deeply infiltrating into the stroma.

Clinical staging

The clinical stages of cervical cancer was based on classification used by International Federation of Gynaecologists and Obstetricians (FIGO) and was done by senior residents or specialists of the department of gynaecology following the already established procedures as follows:-

Stage 0: Preinvasive carcinoma (intraepithial carcinoma, carcinoma in situ)

Stage I: Carcinoma strictly confined to the cervix (extension to the corpus should be disregarded).

Stage IA: Invasion diagnosed only by microscopy, but no greater than 5 mm in

depth from the base of the epithelium, either surface or glandular, from which it originates. Vascular space involvement, venous or lymphatic, should not alter the stage. All grossly identifiable lesions are stage IB.

*Stage IA1: Measured invasion of stroma no grater than 3.0 mm in depth and no wider than .0 mm.

*Stage IA2: Measured invasion of stroma greater than 3.0 mm and no greater than 5.0 mm and no wider than 7 mm.

*Stage IB: Clinical lesions confined to the cervix are pre-clinical lesions of greater dimensions than stage IA2 regardless of whether seen clinically. Preformed space involvement should not alter the stage.

Stage IB1: Clinical lesions no greater than 4.0 cm in size.

Stage IB2: Clinical lesions greater than 4.0 cm in size.

Stage IIA: Invasive carcinoma that extends beyond the cervix, involving the upper two thirds of the vagina

Stage IIB: Invasive carcinoma that involves the upper two thirds of the vagina, with parametrial infiltration that has not reached the pelvic side wall.

Stage III: Invasive carcinoma that extends to either lateral pelvic wall and/or the lower third of the vagina and/or hydronephrosis or nonfunction of kidney due to tumour.

Stage IV: Invasive carcinoma that involves the mucosa of the urinary bladder and/or rectum or extends beyond the true pelvis.

* For the purpose of this study this detailed classification of stage I was not followed it ended at stage Ia, non clinical lesion beyond basement membrane and Ib clinically demonstrable lesion.

The diagnosis of both stages IA1 and IA2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion.

As a rule, it is impossible to estimate clinically whether a cancer of the cervix has extended to the corpus. Extension to the corpus should therefore be disregarded.

Ethical issues

Informed consent was sought from the patient and Ethical Clearance committee of Muhimbili National Hospital. The patients were informed of what was actually going to be done on the study and seek their consent. Pre and post counseling was also done (see appendix B & C).

Limitation of the study

The questions on age at first intercourse and other sensitive questions may not be remembered and answered appropriately especially by women who were old (60 years and above) and may have some little influence on the results on specific variables, likewise staging of cervical cancer was done by senior residents in the department of gynaecology as well as some specialists. This range of experiences might have influenced, though little, the staging of cervix cancer. Staging of cervix cancer by cystoscopy proctosigmoidoscopy and intravenous urography (IVU) was also not done due scarcity of the instrument at MNH. This might have led to lower or upgrade the

stages seen in this study and thus have some influence in the results, though not significantly.

RESULTS

Number of patients

A total of 224 patients who were clinically suspected as having cervical cancer admitted in the gynaecological wards at Muhimbili National Hospital (MNH) from February 2001 to February 2002 were analyzed. Out of these 193 (86.2%) were histologically diagnosed as having cancer while 31 (13.8%) women had non cancerous lesions indicating that clinical suspicion was highly predictive of cervical cancer.

Age Distribution

The age distribution of patients with different type of cervix cancer is shown in figure 1. The age ranged from 24 to 82 years with a peak age in the 40 – 49 years age group. The overall mean age was 49.6 years. Thirty seven (19.2%) were below the age of 40 years.

Geographical distribution

The geographical distribution of women with cervical cancer was analysed based on the areas they resided in the past five years prior to admission in the hospital (Figure 2). The regions which have similar geographical climate and bordering each other were grouped into one zone. By this grouping the country was divided into a total of seven zones shown with their frequencies of cervical cancer as follows:

North eastern zone 23 (14.2%), lake zone 20 (12.3%), western zone 18 (11.1%), central zone 15 (9.3%), eastern zone 65 (40.1%), south western zone 11 (6.8%), southern zone

Marital status:

Figure 3 shows the marital status of women in the study group. There were 124 (77.5%) married women at the time of diagnosis while 8 (5%) were not married and 28 (17.5%) had unstable marital status, that is they were either divorced (19), separated (2) or widowed (7).

Number of wives per husband

Ninety nine (66.4%) of women were correspondingly married to one husband while in the remaining 50 (33.6%) more than one women were married to one husband. Table 3 shows that there was no statistically significant difference between cases and controls in number of wives to a husband ($P < 0.70$).

Sexual – related risk factors for cervical cancer

Table 2 shows mean ages at menarche, at first intercourse, marriage and pregnancy. Age at menarche ranged from 11 – 25 years with a mean of 14.95 years, while age at first exposure to sexual intercourse ranged from 11 to 25 years with a mean at 14 years. The age at first marriage ranged from 11 to 35 years with a mean age of 17.8 years. Out of 161 women who were married at the time, 71 (44%) were below 18 years.

Gravidity and parity:

Frequency distributions of gravidity and parity are shown in figures 4 and 5.

The number of pregnancies ranged from 1 – 15 with mean of 7 pregnancies. One hundred thirty six (81.4%) women had more than 4 pregnancies each. The number of children ranged from 1 – 12 with a mean number of 5.4. One hundred and seven (65.2%) women had more than 4 children each. There was no overall statistically significant

difference in the number of pregnancies (p<0.90) and number of children (p <0.30) between cases and controls except when number of pregnancies reached 7, (p <0.05, 8,(p< 0.001) and 10 (p<0.01) respectively

Stages of cervix cancer

Table 4 shows the distribution of various clinical and pathological stages of cervix cancer. Out of 193 women with cervix cancer, 190 were staged. Out of these 100 women (52.6%) were in stage II, 60 (31.6%) in stage III, 18 (9.5%) in stage I, 11 (5.8%) in stage IV and, only 1 (0.5%) in stage zero. The mean stage at the time of diagnosis was II. However, only 19 (10.0%) women were diagnosed when the disease was in early stages (0 to I) and 170 (90%) when the disease was in late stages (II to IV).

Histological types of cervix cancer

Figure 6 shows the various histological types of cervix cancer. It was found that out of 193 cases of cervix cancer, 167 (86.5%) women had squamous cell carcinoma (Figure 7a) and out of these 13 (7.8%) were of small cell type, while 12 (6.2%) had adenocarcinoma (Figure 7b). Other rare types of cervix cancer included adenosquamous carcinoma 4 (2.1%) (Figure 7c), adenoid cystic carcinoma 2 (1%) (Figure 7d), undefferentiated small cell carcinoma 6 (3.2%) (Figure 7e), and carcinoid tumour 2 (1%). (Figure 7f) The mean ages of these histological types of cervical cancer were as follows: squamous cell carcinoma (50 years), adenocarcinoma (53 years), adenosquamous carcinoma (46 years), adenoid cystic carcinoma (54 years) and small cell carcinoma (54 years). This indicates that small cell carcinoma occur at a relatively older age compared to squamous cell carcinoma.

Small cell carcinoma of the cervix:

Figure 8 shows grades and subtypes of small cell carcinoma of the cervix. There were 21 women who had small cell carcinoma. This represented 10.9% of all carcinomas of the cervix. Two histological grades of small cell carcinomas were described during the study. These included 13 (61.9%) squamous cell carcinoma, small cell non-keratinizing type and 6 (28.6%) undifferentiated small cell carcinoma. The rest 2 (9.5%) of the tumours composed of carcinoid tumours. The overall mean age of small cell carcinoma of the cervix was 54.4 years. However, the mean ages of each grade of small cell carcinoma were 53.5 years for poorly differentiated squamous cell carcinoma, 50.2 years for undifferentiated carcinoma and 68 years for carcinoid tumour. The clinical stages (FIGO) of all 21 small cell carcinoma (Table5) ranged from II to III. Nine (42.9%) in stage II. Twelve (57.1%) were in stage III. There was no statistically significant differences between small cell carcinoma and non-small cell carcinoma in all stages of cervix cancer ($P < 0.90$)

HIV – 1 Serostatus

Table 6 shows the distribution of serostatus of patients who were tested for HIV – 1. Out of 224 patients who were recruited for the study, 187 gave consent for their blood to be tested for HIV. Out of these 21 (11.2%) were HIV positive while 166 (88.8%) were negative. The age range of those tested was from 25 to 56 years with a mean age of 41.5 years

T- lymphocyte counts

Table 7 shows T-cell count and clinical staging of HIV – 1 positive patients with cervix cancer. Lymphocyte count ranged from 61 to 850 cells/mm3 for CD+4 cell while the CD4/ CD8 ratio ranged from 0.07 to 0.96. The mean values for the T-lymphocyte subsets were 282 cells/mm3 for CD4+ and 1047 cell/mm3 for CD8+. The mean CD4/CD8 ratio was 0.32. The CD4+ lymphocyte count was below 500 cells/mm3 in 10(83.3%) of all HIV positive patients who had cervical cancer. The difference of the CD4+cell count was statistically significant between those who were HIV seropositive and had cervical cancer compared to those who were HIV seronegative without cervical cancer ($p<0.05$). This means that cervical cancer is associated with pronounced immuodeficiency, as in other HIV-related cancers.

Clinical staging

All 13 patients who were HIV positive had squamous cell carcinoma and out of these 2 (15.4%) were in stage I, 9 (69.2%) were in stage II, and the other 2 were in stage III and IV each. Table 8 shows the distribution of clinical staging of cervix cancer among HIV positive cases and controls. The age range of 13 HIV-1 positive patients who had squamous cell carcinoma was 35 – 56 years with a mean of 43.5 years. There was no statistically significant difference between clinical stages of patients who had cervix cancer and were HIV positive and controls ($p<0.10$).

Non-cancerous lesions of the cervix

Table 9 shows 31 noncancerous lesions which were histologically diagnosed although the patients were clinically suspected as having cervix cancer. The lesions represented

13.8% of all 224 cervical lesions which were clinically suspected as cervix cancer in the studied patients. These non-cancerous lesions included 15 (48.4%) non – specific chronic cervicits (NSCC), 6 (19.4%) endocervical polyps, 5(16.1%) cervical intraepithelial neoplasia (CIN) lesions, 4 (12.9%) granulomatous lesions, 1 (3.2%) tuberculosis (Figure 9), 3(9.7%) schistosomiasis (Figure 10) and one case of condyloma accuminatum.

Cervical lesions in HIV positive women

Table 10 shows the distribution of cervical lesions diagnosed in HIV positive cases. Out of 21 patients who were HIV positive, 13 (61.9%) had squamous cell carcinoma (SCC), 5(23.8%) non – specific chronic cervicitis (NSCC), 2 (9.5%) cervical intraepithelial neoplasia (CIN) and 1 (4.8.%) tuberculosis of the cervix. No small cell carcinomas were encountered among the HIV positive cases.

Grimelius stain and Immunohistochemistry

Table 11 shows reactivity of 21 subtypes of small cell carcinoma of the cervix with Grimelius stain and NSE. Out of 21 small cell carcinomas, 8(38.1%) showed reactivity with Grimelius. The staining was confined to cell cytoplasm which appeared as brown colour and nuclei as brown / black (Figure12a). Seven tumours (33.3%) showed immunoreactivity to NSE (figure 12b) indicating neuroendocrine differentiation of the tumours. Tumours showed varying intensity of staining from focal(+) to diffuse (+++) staining.

Five monoclonal antibodies were applied on tissue sections in order to verify the presence of the following antigens in the tissue:- Neuronespecific enolase (NSE), Human papillomavirus (HPV), Epithelial membrane antigen (EMA) and Ki-67.

Table 12 shows the results of immunostaining with anti-HPV antibody (polyclonal, clone L1). Out of 21 patients, 13 were HIV positive and out of these 9 (69.2%) patients expressed HPV (Figure 13) while 4 (30.8%) did not. This difference was not statistically significant ($p<0.10$).

Anti-EMA clone E29. Mab (DAKO) was used to aid in the identification of cells of epithelial lineage. Table 13 shows immunoreactivity reactivity of EMA on small cell and non-small cell cervical carcinoma. Twenty (95.2%) out of 21 small cell carcinoma and all 21 (100%) of non-small cell carcinomas (Figure 14) stained positively with EMA, though in varying intensity, indicating epithelial lineage for both types of tumour.

Table 14 shows that sixteen of 21 non-small cell carcinoma (76.2%) expressed HPV while 5(23.8%) did not express. In general there was more HPV expression in non-small cell carcinoma than small cell carcinoma. There was a statistically significant difference between patients with small cell carcinoma and those with non small cell carcinoma on expression of HPV ($P <0.004$), regardless of HIV serostatus. Eight (61.5%) of the 13 patients with non small cell carcinoma who were also HIV-seropositive expressed HPV while 5(38.5%) did not.

Table 15 shows the percentages of proliferative index, of Ki-67 in percentage on both small cell and non-small cell carcinomas (Figure 15). The mean proliferative index of each was 38% for small cell carcinoma and 25% for non-small cell carcinoma.

There was a statistically significant difference on proliferative index between the two groups of carcinomas ($p<0.001$) meaning that small cell carcinoma is more aggressive.

Relationship between CD4+ cell counts, HIV and HPV

Table 16 shows that CD4+ lymphocyte count in HIV-seropositive patients who also expressed HPV was below 300/mm^3 in 8 (61.5%) except one who had a slightly higher count (340/mm^3). Four (36.8%) of the HIV–seropositive patients were HPV negative and their CD4+ lymphocyte counts were above 300/mm^3. Seven (77.8%) of the nine HIV–seropositive patients who had HPV expression presented in advanced clinical stage of cervix cancer (stage II – IV) and their CD4+ lymphocyte count were very low (ranged from 61/mm3 – 340/mm^3) with a mean of 181/mm^3. One of them had clinical stage IVA and the lowest CD4+ lymphocyte count (47/mm^3). Four (30.8%) of the 13 seropostitive patients were HPV negative and their CD4+ lymphocyte count ranged from 208/mm^3 to 850/mm^3, with a mean of 560/mm^3, a slightly moderate immunosupression. The clinical stages of the disease in the HPV negative but HIV-seropositive patients was II in all 4 patients. Out of 21 (all HIV-seronegative) patients with small cell carcinoma, 10 (47.6%) expressed HPV (Figure 14), while 11(52.4%) did not show any HPV immunoreactivity.

DISCUSSION

This study shows that out 224 patients studied, 192 (99.5%) had invasive cancer and only one (0.5%) had carcinoma in situ, an indication that most patients are diagnosed late. These findings of late presentation of cervical cancer has been reported before in developing nations[37] and thought to be due to lack of medical facilities such as screening methods by Pap-smear coupled with ignorance. The finding of a wide age range distribution of cervix cancer from second to eighth decade is unique to this study. However, the overall mean age of 49.6 years is similar to those reported before[37,38].

One of the most interesting aspects of cervix cancer studies is the variability in the disease pattern occurring in different communities at different geographical areas[21,39]. The present study has shown similar variable distribution in Tanzania. However, the observed differences of cervix cancer distribution in different zones and regions in Tanzania cannot be reasonably explained. It suffices to speculate that multifactorial pathogenic factors may be operative in the different zones dictating the variable distribution of the disease in the country.

With possible exception of breast cancer no female – limited tumour has generated as much speculation regarding the aetiology as carcinoma of the cervix. Marriage perse cannot cause cervical cancer but may be an associated risk factor as shown that 77.5% of women were married at the time of diagnosis in this study and 44% were married before 18 years.

Further more, general experience show that in Dar es Salaam and in Tanzania at large early marriage (and therefore, early initiation of sexual life) is customary in many parts

of this country[40]. Women who marry before the age of twenty have approximately a two fold greater risk of developing invasive cervical cancer compared to women who marry later[41]. In this study 44% of patients were married at an age below 18 years and thus it might be inferred that they were also at an increased risk compared to women who married later.

Sexual – related risk factors of cervical cancer which included age at first intercourse, menarche, marriage and pregnancy have been reported before as Gagnon[42] noted the rarity of the disease in virgins, attenting shifted from marriage to initiation of sexual relations. In his studies, age at first coitus proved to be a stronger correlate of risk for cervical cancer than age at first marriage. Women who have first coitus before the age of twenty have between a two and three fold increased risk for invasive cancer compared to women who start sexual relations later indicating a progressive increase in the risk with earlier age at first coitus[42]. In the present study age of exposure to sexual relations ranged from 11 to 25 years with a mean age of 14 years. This shows that majority of our patients had first coitus before the age of twenty and therefore were at increased risk for cervical cancer and subsequently developed invasive cancer.

Another risk factor is the number of sexual partners, often indexed by multiple marriages, separations, and divorces. Women with multiple marriages or partners had approximately a two or three fold increased risk for cervical cancer compared to women with one partner or marriage[43,44]. In this study (17.5%) of women had unstable marital status, that is they were either divorced, separated or widowed and thus were at an increased risk of developing the disease. In addition 33.6% of women had multiple

marriages. Early age at first coitus and multiple consorts are two of the strongest personal risk factors for cervical cancer all of which allow early and prolonged exposure to an infectious agent(44). The importance of age at first coitus may also derive from the susceptibility of the adolescent cervix to atypical transformation. The observed strength could vary with the population studied. In population where the prevalence of genital infection is high, age at first coitus may appear more important. In population where prevalence of genital infection is low, number of partners might appear stronger(44). In any case the two variables tend to be correlated, and together they account for a number of other reported risk factors(45) .

With regard to number of pregnancies and deliveries, this study shows that women had an average of 7 pregnancies and 5 deliveries. However, there was no statistically significant difference in the number of pregnancies ($p<0.90$) and number of children (<0.30) between cases and controls, although when the number of pregnancies reached 7 and above it was statistically significant ($p<0.05$). This suggests that the risk of developing cervical cancer increases after seven pregnancies. However, it is a known fact that early marriage predisposes women to early pregnancy and greater number of pregnancies. This fact is also supported by the finding in this study that the average number of pregnancies in cervical cancer patients were seven with 44% of women being married at an early age (before 18 years). This also confirms other studies that early age at first pregnancy and number of completed pregnancies increases the cervical cancer risk

Various stages and depth of invasion are well correlated with prognosis in patients with

Various stages and depth of invasion are well correlated with prognosis in patients with cervix cancer[41]. Early stages of cervix cancer (stage 0 and I) are associated with good prognosis compared to late stages (stages II – IV) which give poor prognosis. The findings that 52.6% of the patients studied were in stage II, 31.6% in stage III and 5.8% in stages IV indicate that 90% the patients had advanced disease similar to what has been reported before[41] It can therefore be concluded from this study that the majority of the patients at Muhimbili National Hospital are diagnosed when the disease is at an advanced stage with poor prognosis and a low – five year survival rate. The high incidence of invasive cancer at MNH and consequently mortality rate of the disease indicate lack of effective screening programmes or ignorance.

The predominance of squamous cell carcinoma accounting for 86.5% in this study is in agreement with most studies[41]. Likewise adenocarnoma accounted for 6.2%, which is similar to that reported by others[46,47]. Again the average age of 53 years for invasive adenocarcinoma support other reports[46] that adenocarcinoma tends to occur in a slightly older population than squamous cell carcinoma (average 50 years).

Small cell carcinoma of the cervix represents an uncommon variant of cervix cancer with particularly poor prognosis[23]. The term "small cell carcinoma" has been adequately defined[26] . Small cell carcinoma accounted for 10.9% of all cervical cancers in the current study, the finding is slightly higher than what has been reported before[23,48]. Of the small cell cervical carcinomas, squamous cell carcinoma, small cell non-keratining type was the predominant type (61.9%) besides undifferentiated carcinoma and N.E carcinoma. The findings are similar to what has been reported[24,49]

carcinoma and neuroendocrine carcinoma. The findings are similar to what has been reported[24,49]

Small cell carcinoma is usually seen in younger women and has a more aggressive clinical course[25,26]. It was seen in older women (mean age 54 years) although there was no statistically significant difference in the clinical stage between cases and controls (those with non-small cell carcinoma) (<0.90). Of the 21 small cell carcinomas in this study, 8 (38.1%) showed neuroendocrine differentiation on staining with Grimelius and 7 (33.3%) on staining the same cases sing NSE, indicating that 8 (38.1%) were neuroendocrine carcinomas. This is slightly higher than those reported in other series[25,27,64]. In this study small cell NE carcinomas were all in advanced clinical stages of the disease (stage II to IV), probably an indication of aggressive clinical nature concordant to other studies. In some studies, these tumours secreted detectable levels of various hormones[27,50]. This was not investigated in this study but it could be of interest in future as a follow up.

Human papillomavirus (HPV) type 18 is found in most small cell carcinoma and likewise HPV16 has also been reported[28,71]. This is supported by this study where 47.6% of small cell carcinoma were found to express HPV. The fact that 61.9% of all cervical cancers expressed HPV indicates a significant role played by HPV in the pathogenesis of cervical cancer. All patients with small cell carcinoma were HIV seronegative but 10 (47.6%) of these patients expressed HPV. This indicates that there are other factors responsible for HPV expression in cervical cancer, apart from immunosuppression and HIV tat protein known to up-regulate HPV expression[33].

However, the risk of developing cervical disease in association with HPV is clearly higher in the setting of HIV infection, and there are suggestions that the risk increases as the degree of HIV associated immunosuppression increases[33] .The association of HIV and small cell carcinoma has not been conclusively studied and there are no reports from Tanzania. In this study out of the 21 small cell carcinoma encountered, there was no patient who was HIV-infected. Further follow-up studies are needed to make conclusions on this.

Studies on the association of HIV, HPV and CD4+ cell count in women with cervical cancer have found that he prevalence of HPV increases with CD4+ cell count from 23% among immunocompetent HIV negative subjects to 45% in mild or moderate immunosuppression[4,72]. It was then concluded that the risk of developing cervical cancer in association with HPV is higher in the setting of HIV infection, and increases as the degree of HIV-associated immunisuppression increases[4]. In this study the mean $CD4^+$ cell count in HPV positive; HIV-immunosuppressed patients was 181/mm^3 (16%) while the mean in HIV – seronegative patients was 560/mm^3(22%), supporting the notion of others[4,72] that the prevalence of HPV increases with decreasing CD4+ cell count. There are several reasons which can explain failure of HPV expression in 38.8% of the HIV seropositive patients in this study. One which is most unlikely could be that there was no HPV in the tumours. Other reasons include sensitivity of the immunoperoxidase staining method which was used, the cross linking of antigens caused by formalin fixation and the effectiveness of the antigen retrieval method employed[51]. Also there could be a down-regulation of HPV L1 or E6/E7 genes expression by

Several cell cycle–specific nuclear antigens have been recognized using immunohistochemical methods, allowing a reliable evaluation of tumour growth fraction. In particular, Ki-67 is a nuclear antigen which is recognized by various clones of monoclonal and polyclonal antibody Ki-67[54,71], typically expressed in proliferating cells during the G1, S, G2 and M phases of the cell cycle[54] thus useful in a number of human neoplasm providing information about cellular proliferation rate and, therefore prognosis. In this study the mean proliferation was 30% tumour cells in small cell carcinoma and 25% in non-small cell carcinoma as determined by Ki-67, regardless of the HPV and HIV status. This was statistically significant between the two carcinomas ($p<0.001$) and agrees with other studies[54] meaning that small cell carcinoma is more aggressive and may have a poor prognosis.

Non-concerous lesions of the cervix

This study has shown that a proportion (13.9%) of lesions which are clinically diagnosed as cervical cancer are in fact non-cancerous lesions. It therefore follows that non-specific chronic cervicitis (NSCC) (48.4%), granulomatous lesions which included schistosomiasis (9.7%) and tuberculosis (3.2%) of the cervix may be misdiagnosed for cervical carcinoma if confirmatory histology is not done. Cervical TB may be acquired by haematogenous, lymphatic spread or by direct extension[7,55,56]. In rare cases, cervical TB may be a primary infection,[7,55,56] introduced by a partner with genital tuberculosis. The incidence of TB has increased recently which is partly attributable to HIV pandemic[56] . There should therefore be a high index of suspicion of tuberculosis in women with an abnormal cervical appearance, especially from areas where HIV and TB

provide patients with small cell carcinoma of the cervix the best chance of cure[25]. It is important to diagnose correctly and differentiate neuroendocrine carcinomas of the cervix from other carcinomas of this location, so as to plan effective curative management. Currently this is not done at Muhimbili National Hospital. Light microscopy of H&E which is used for the diagnosis of cervix cancer cannot differentiate small cell neuroendocrine carcinomas of the cervix from other carcinoma and therefore effective curative management is not possible. It is recommended that all such tumours should be further subjected to Grimelius histochemistry and immunohistochemistry for NSE to pick up small cell NE carcinomas for appropriate management.

CONCLUSION

Cervical cancer is common at MNH and Tanzania at large and is a major problem in females and mostly in the active age group. It is also reported at advanced stage, depth of invasion and tumour size all of which are poor prognostic factors. Age at first coitus, early age at marriage and multiple pregnancies were sexual – related risk factors which are pointers of sexual activity and found to be important in the pathogenesis chain of cervix cancer.

Majority of the patients presents with the disease at an invasive and advanced clinical stages, leading to high mortality rate of the disease in the society which is an indicator of lack of screening programmes. This fact underlies the need for urgent screening programmes at a national level.

Small cell carcinoma is not uncommon at Muhimbili National Hospital and Tanzania at large. Relative to other cell types, small cell carcinoma of the cervix is an aggressive

neoplasm. Thus small cell carcinoma needs special treatment with multimodality therapy, combining radical surgery and radiation with cytotoxic chemotherapy, as these may provide patients with best chance for cure. It is also important to recognize the neuroendocrine component as this type of carcinoma requires special therapeutic consideration.

RECOMMENDATION

The common denominator in the aetiology of cervical cancer is sexual activity. It is recommended that preventive measures and age at first marriage and pregnancy should be enforced such that marriage should be at least 20 years since it is known that early coitus is associated with increased risk of cervical cancer. Further, education on family planning should be encouraged so that the number of children should not exceed 6. As a matter of policy, the Tanzanian government should encourage cervical cancer screening by Pap smear to sexually active women from 20 years and above at three years intervals so as to reduce to a minimum the incidence of cervical cancer cases in the country.

For correct management of small cell NE carcinoma, accurate diagnosis supplemented by histochemistry and immunohistochemistry must be done.

Table 1. A list of antibodies used in the study.

S/N	Antibody	Clone	Specificity	Source
1.	Anti-Neuron specific Enolase (Mouse Anti Human)	BBS/NC	Labels cells of Neuroendocrine origin	DAKO
2.	Ki67	S5	Expressed in all human proliferating cells (in normal as well as tumour cells)	DAKO
3.	Anti-HPV	K1H8	Human Papillomavirus	DAKO
4.	* Anti- HPV	L1	Human Papillomavirus	DAKO
5.	Anti-Human EMA	E 29	1. Normal and neoplastic epithelium, especially glandular epithelium	DAKO
6.	Mouse Anti-human T cell, CD4, Helper/inducer	SK3	Helper/Inducer Subpopulation of T cells	Becton an Dickson (BD)
7.	Mouse Anti-human T cell, CD8, Suppressor/Cytotoxic	SK1	Suppressor/Cyto-toxic subpopulation to T cells	Becton and Dickson (BD)

* Polyclonal Antibody

TABLE 2: Number of wives to one husband among patients with cervical cancer and controls

Number of wives	Frequency		x^2	p - value
	Cases	Control		
2	33	28	0.89	< 0.5
3	11	12	0.08	< 0.70
4	4	3	0.08	< 0.70
5	2	1	0.08	< 0.70
Total	**50**	**44**	**0.36**	

X^2 = Chi – square

Table 3. Sexual – related risk factors of cervical cancer at first exposure

Risk factor	Age Range		Mean Age	
	Cases	Control	Case	Control
Marriage	11 – 35	20 – 30	18	22
Pregnancy	13 – 45	18 – 28	19	20
Sexual intercourse	11 - 25	18 - 26	14	20

Table 4. Age distribution of various clinical and pathological stages of cervix cancer

Age group	Stages					Total
	0 (%)	I (%)	II (%)	III (%)	IV (%)	
< 20	-	-	-	-	-	-
20 – 29	-	1 (5.6)	2 (2)	1 (1.7)	0 (0)	4
30 – 39	-	5 (27.8)	16 (16)	11 (18.3)	2 (18.2)	34
40 – 49	1 (100)	5 (27.8)	40 (40)	18 (30)	5 (45.4)	69
50 – 59	-	3 (16.6)	18 (18)	11 (18.3)	2 (18.2)	34
50 – 69	-	2 (11.0)	16 (16)	14 (23.3)	1 (9,1)	33
70 – 79	-	1 (5.6)	6 (6)	5 (8.4)	1 (9.1)	13
≥ 80	-	1 (5.6)	2 (2)	0 (0)	0 (0)	3
TOTAL	**1**	**18**	**100**	**60**	**11**	**190**

Table 5: Clinical stages of 21 small cell carcinoma and non-small cell carcinoma of the cervix

Stage	Number		%	X^2	P value
	SMCC	NOSCC			
I	0	2	0	2.0	< 0.90
II	9	15	42.9	0.4	< 0.90
III	12	4	57.1	2.0	<0.90
IV	0	0	0	0.0	-
Total	**21**	**21**	**100**		

X^2 = Chi Square

SMCC: Small Cell Carcinoma

NOSCC: Non Small Cell Carcinoma

Table 6: HIV – 1 Serostatus in women with small cell and non small cell carcinoma

SN	Small cell carcinoma	HIV Serostatus	Non small cell carcinoma	HIV Serostatus
1.	SCC, Small cell type	-	SCC	+
2.	SCC, Small cell type	-	SCC	+
3.	SCC, Small cell type	-	Verrucous carcinoma	+
4.	Neuroendocrine carcinoma	-	SCC	+
5	SCC, Small cell type	-	SCC	+
6.	SCC, Small cell type	-	SCC	+
7.	SCC, Small cell type	-	SCC	+
8.	Undifferentiated small cell carcinoma	-	SCC	+
9.	SCC, Small cell type	-	SCC	+
10	Undifferentiated small cell carcinoma	-	SCC	+
11.	SCC, Small cell type	-	SCC	+
12.	Neuroendocrine carcinoma	-	SCC	+
13.	Undifferentiated small cell carcinoma	-	SCC	+
14.	Undifferentiated small cell carcinoma	-	Adenocarcinoma	-
15.	SCC, small cell type	-	Adenocarcinoma	-
16.	Undifferentiated small cell carcinoma	-	Undifferentiated carcinoma	-
17.	SCC, small cell type	-	SCC	-
18.	SCC, small cell type	-	Adenocarcinoma	-
19	Undifferentiated small cell carcinoma	-	Adenocystic Carcinoma	-
20.	SCC, small cell type	-	SCC	-
21.	Undifferentiated small cell carcinoma	-	SCC	-

SCC = Squamous cell carcinoma

++ Focal, intense staining

+ Focal, weak staining

Table 7: T-lymphocyte count and clinical staging of HIV positive patients with cervix cancer

Stage	No. of Cases	Mean Age	Mean CD4 %	Mean CD8 %	Mean $CD4/mm^3$	Mean $CD8/mm^3$	Mean CD4/CD8 ratio
I	1	35	5	70	131	1827	0.07
II	9	44.6	23	40	263	955	0.27
III	2	43.5	22	38	566	1565	0.06
IV	1	40	9	17	75	141	0.53

Table 8: Distribution of clinical staging of cervix cancer among HIV positive cases and controls

Stage	Case (%)	Control (%)	X^2	p value
I	2 (15.4)	3 (23)	0.33	0.95
II	9 (69.2)	5 (38.5)	3.2	0.10
III	1 (7.7)	5 (38.5)	3.2	0.10
IV	1 (7.7)	0	0.0	
Total	**13**	**13**		

Table 9. Histological diagnosis of non-cancerous lesions of the cervix

S.N.	Lesions	Number	%
1	*NSCC	15	48.4
2	Schistosomiasis	3	9.7
3	Tuberculosis	1	3.2
4	*CIN	5	16.1
5	Endocervical polyp	6	19.4
6	Condyloma accuminatum	1	3.2
	TOTAL	**31**	**100**

* NSCC = Non – specific chronic cervicits.
* CIN = Cervical intraepithelia neoplasia.

Table 10. Distribution of cervical lesions diagnosed in HIV positive cases

S.N.	Lesions	Number	%
1.	Squamous cells carcinoma	13	61.9
2.	Non-Specific chronic cervicitis	5	23.8
3.	Cervical intraepithelial neoplasia	2	9.5
4.	Tuberculosis	1	4.8
	Total	21	100

Table 11. Reactivity of small cell carcinoma of the cervix for grimelius (gm) stain and neuron specific enolase (NSE)

S.N.	Histological Type of cancer	Reactivity	
		GM	NSE
1.	SCC, Small Cell type	++	++
2.	SCC, small cell type	-	-
3.	SCC, small cell type	+	-
4.	Carcinoid tumour	+	+
5.	SCC, small cell type	-	-
6.	SCC, small cell type	-	-
7.	SCC, small cell type	-	-
8.	Undifferentiated small cell carcinoma	-	-
9.	SCC, Small cells type	++	+
10.	Poorly differentiated small cell carcinoma	+	+
11.	SCC, Small cell type	-	-
12.	Malignant carcinoid tumour	+	+
13.	Poorly differentiated small cell carcinoma	+	+
14.	Poorly differentiated small cell carcinoma	-	-
15.	SCC, small cell type	-	-
16.	Undifferentiated small cell carcinoma	-	-
17.	SCC, small cell type	-	-
18.	SCC, small cell type	-	-
19.	Poorly differentiates cell carcinoma	-	-
20	SCC – Small cell type	-	-
21.	Undifferentiated small cell carcinoma	+++	++

+++ = Positive, diffuse reaction ++ = Positive, intense, but focal

\+ = Positive, mild reaction but focal - = Negative

NSE = Neurone specific enolase GM = Grimelius

Table 12: HPV expression in HIV-seronegative women with small cell carcinoma

SN	Small cell carcinoma	HIV Serostatus	HPV*
1.	SCC, Small cell type	-	-
2.	SCC, Small cell type	-	-
3.	SCC, Small cell type	-	++
4.	Neuroendocrine tumour	-	-
5	SCC, Small cell type	-	+
6.	SCC, Small cell type	-	+
7.	SCC, Small cell type	-	+
8.	Undifferentiated small cell carcinoma	-	-
9.	SCC, Small cell type	-	+
10	Undifferentiated small cell carcinoma	-	-
11.	SCC, Small cell type	-	+
12.	Neuroendocrine carcinoma	-	+
13.	Undifferentiated small cell carcinoma	-	+
14.	Undifferentiated small cell carcinoma	-	-
15.	SCC, small cell type	-	+
16.	Undifferentiated small cell carcinoma	-	-
17.	Scc, small cell type	-	-
18.	SCC, small cell type	-	-
19	Undifferentiated small cell carcinoma	-	-
20.	SCC, small cell type	-	-
21.	Undifferentiated small cell carcinoma	-	-

SCC = Squamous cell carcinoma

++ Focal, intense staining

+ Focal, weak staining

* Immunoperoxidase staining in tissue sections

Table 13: Expression of EMA in small cell and non-small cell carcinoma of the cervix.

SN	SMCC	EMA	NOSMCC	EMA
1.	SCC, Small cell type	++	SCC	+
2.	SCC, Small cell type	++	SCC	+
3.	SCC, Small cell type	++	Verrucous carcinoma	+
4.	Neuroendocrine tumour	+	SCC	+
5	SCC, Small cell type	++	SCC	+
6.	SCC, Small cell type	++	SCC	+
7.	SCC, Small cell type	++	SCC	+
8.	Undifferentiated small cell carcinoma	+	SCC	+
9.	SCC, Small cell type	++	SCC	+
10	Undifferentiated small cell carcinoma	+	SCC	+
11.	SCC, Small cell type	++	SCC	+
12.	Neuroendocrine carcinoma	-	SCC	+
13.	Undifferentiated small cell carcinoma	+	SCC	+
14.	Undifferentiated small cell carcinoma	+	Adenocarcinoma	++
15.	SCC, small cell type	++	Adenocarcinoma	+
16.	Undifferentiated small cell carcinoma	+	Undifferentiated carcinoma	++
17.	SMCC	++	SCC	+
18.	SCC, small cell type	+	Adenocarcinoma	++
19	Undifferentiated small cell carcinoma	+`	Adenocystic Carcinoma	+
20.	SCC, small cell type	++	SCC	+
21.	Undifferentiated small cell carcinoma	+	SCC	+

SCC = Squamous cell carcinoma
++ Focal, intense staining
+ Focal, weak staining

Table 14: HPV expression in HIV-seropositive women with non-small cell carcinoma of the cervix

SN	Type of carcinoma	Serostatus	*HPV
1.	SCC	+	+
2.	SCC	+	+
3.	Verrucous carcinoma	+	+
4.	SCC	+	+
5	SCC	+	+
6.	SCC	+	-
7.	SCC	+	++
8.	SCC	+	-
9.	SCC	+	++
10	SCC	+	-
11.	SCC	+	++
12.	SCC	+	-
13.	SCC	+	+
14.	Adenocarcinoma	-	+
15.	Adenocarcinoma	-	+
16.	Undifferentiated carcinoma	-	+
17.	SCC	-	
18.	Adenocarcinoma	-	+
19	Adenocystic Carcinoma	-	+
20.	SCC	-	+
21.	SCC	-	+

SCC = Squamous cell carcinoma

++ Focal, intense staining

+ Focal, weak staining

* Immunoperoxidase staining in tissue sections

Table 15: Expression of Ki67 in small cell and non-small cell carcinoma of the cervix.

SN	SMCC	*Ki-67(%)	NON-SMCC	*Ki-67(%)
1.	SCC, Small cell type	0	SCC	30
2.	SCC, Small cell type	0	SCC	30
3.	SCC, Small cell type	40	Verrucous carcinoma	0
4.	Carcinoid tumour	0	SCC	0
5	SCC, Small cell type	0	SCC	0
6.	SCC, Small cell type	0	SCC	0
7.	SCC, Small cell type	40	SCC	30
8.	Undifferentiated small cell carcinoma	50	SCC	30
9.	SCC, Small cell type	0	SCC	0
10	SCC, small cell type	50	SCC	0
11.	SCC, Small cell type	30	SCC	20
12.	Malignant Carcinoid tumour	30	SCC	30
13.	Undifferentiated small cell carcinoma	50	SCC	0
14.	Undifferentiated small cell carcinoma	0	Adenocarcinoma	0
15.	SCC, small cell type	50	Adenocarcinoma	0
16.	Undifferentiated small cell carcinoma	30	Undifferentiated carcinoma	0
17.	SCC, small cell type	40	SCC	10
18.	SCC, small cell type	30	Adenocarcinoma	0
19	Undifferentiated small cell carcinoma	35	Adenocystic Carcinoma	0
20.	SCC, small cell type	0	SCC	10
21.	Undifferentiated small cell carcinoma	35	SCC	30
Mean proliferative index		38%		25%

SCC = Squamous cell carcinoma
NOSMCC = non-small cell carcinoma
SMCC = small cell carcinoma
* Ki67 = % positive tumour cells

Table 16: HPV, HIV and CD4+ lymphocyte count in patients with cervix cancer in relation to stage

SN.	Age	Type of cancer	HPV	HIV	CD4+/mm3	Stage
1.	35	SCC	+	+	131	IA
2.	56	SCC	+	+	61	IB
3.	50	SCC	++	+	340	IIA
4.	44	SCC	-	+	668	IIA
5.	40	SCC	-	+	308	IIA
6.	40	SCC	+	+	75	IIB
7.	41	SCC	++	+	187	IIB
8.	41	SCC	-	+	850	IIB
9.	42	SCC	++	+	413	IIB
10.	46	SCC	++	+	79	IIB
11.	41	SCC	+	+	267	IIB
12.	46	SCC	++	+	79	IIIA
13.	43	SCC	+	+	47	IVA

SCC: Squamous cell carcinoma

Figure 1. Age distribution of cervical cancer cases diagnosed at MNH from February 2001 – February 2002

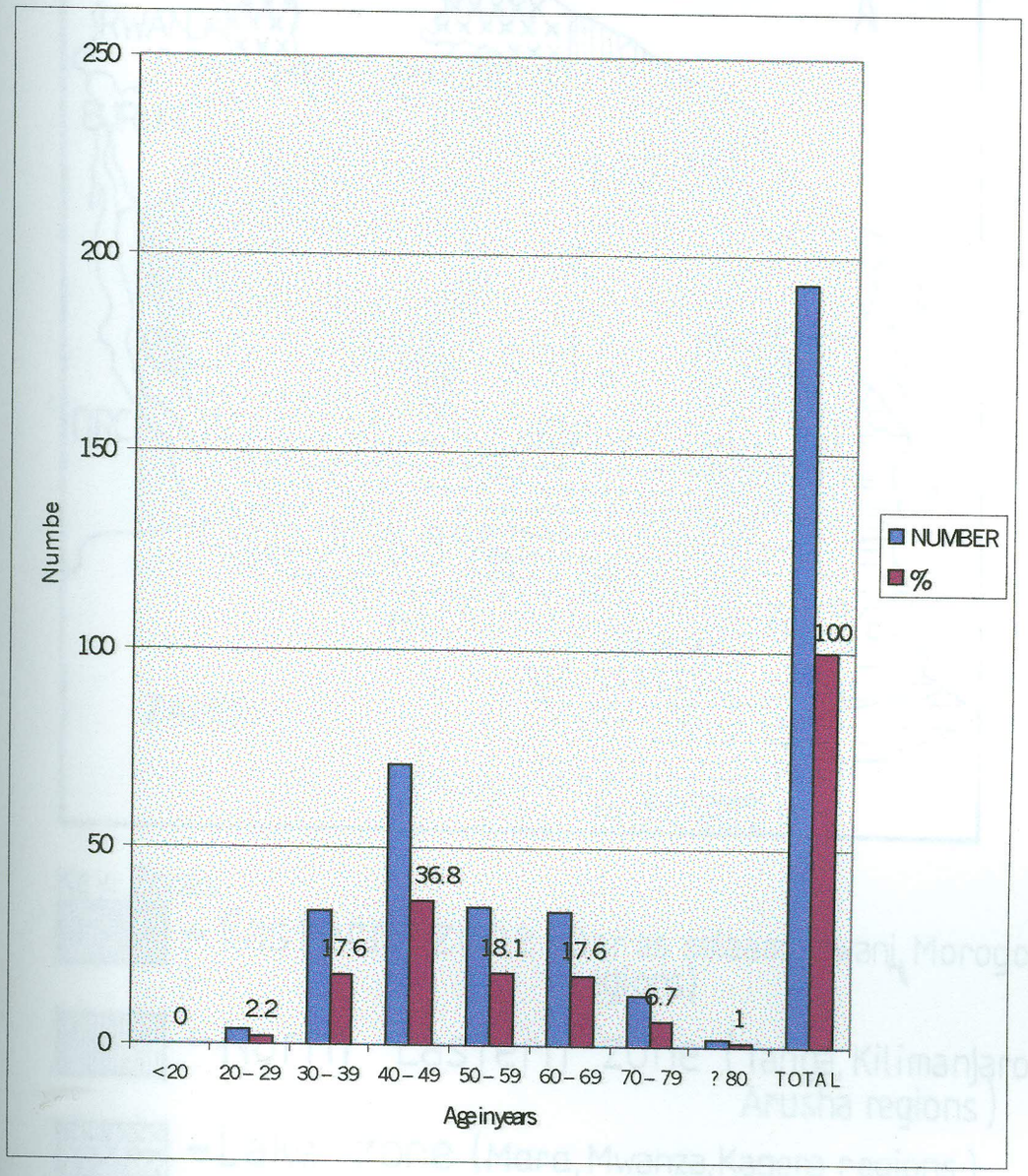
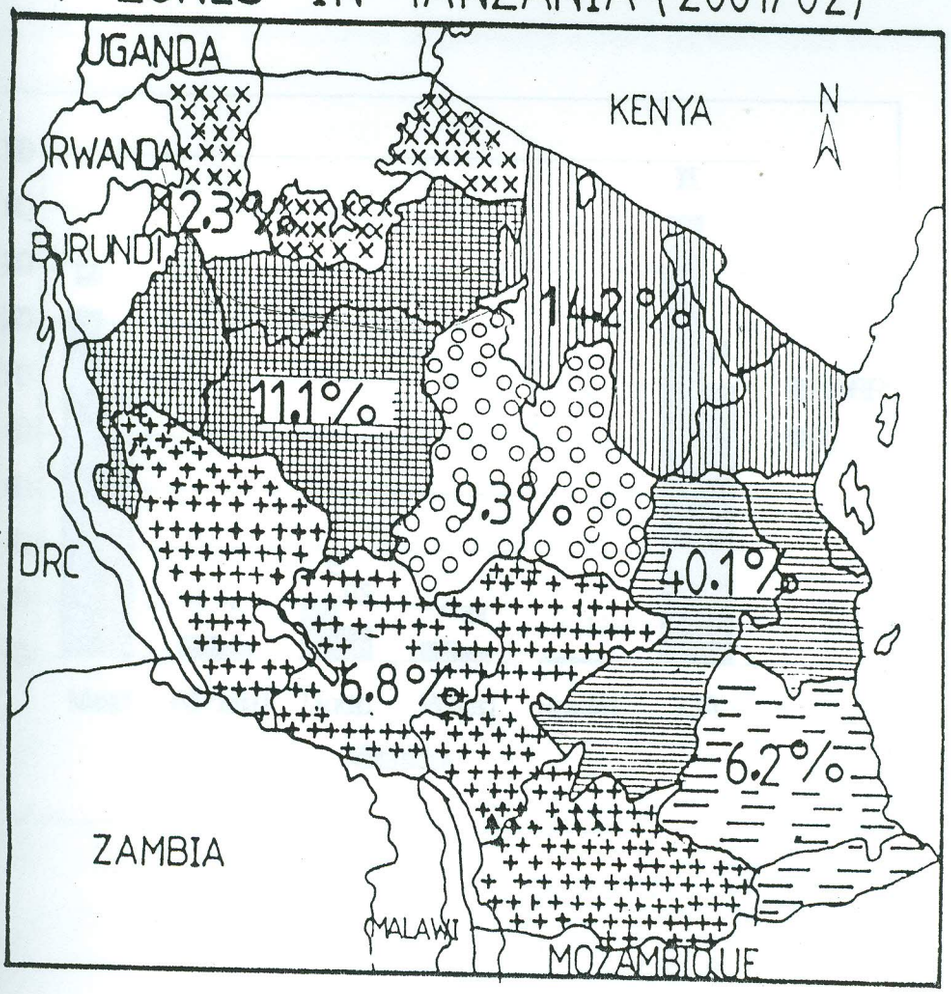


FIGURE 2
DISTRIBUTION OF CERVICAL CANCER IN 7 ZONES IN TANZANIA (2001/02)



Key: Zones

- Eastern zone (Dar es salaam, Pwani, Morogoro, regions)
- North Eastern zone (Tanga, Kilimanjaro, Arusha regions)
- Lake zone (Mara, Mwanza, Kagera regions)
- Western zone (Kigoma, Tabora, Shinyanga regions)
- Central regions (Dodoma, Singida regions)
- South Western zone (Mbeya, Iringa, Rukwa regions)
- Southern zone (Mtwara, Lindi regions)

Figure 3. Marital status of women with cervical cancer

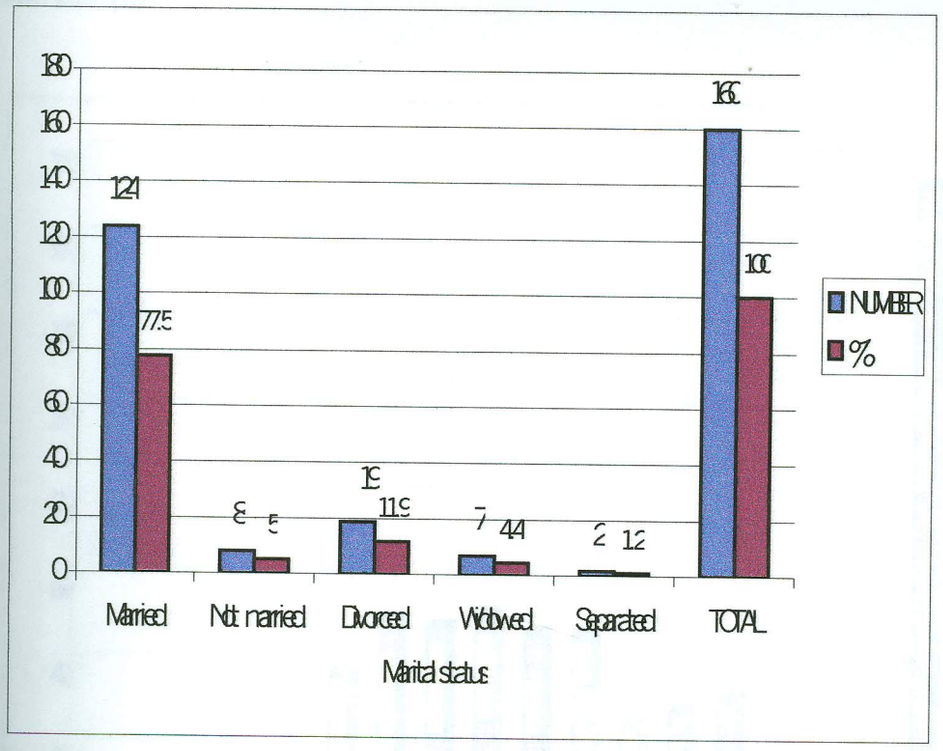


Figure 4. Frequency distribution of gravidity among patients with cervical cancer and controls

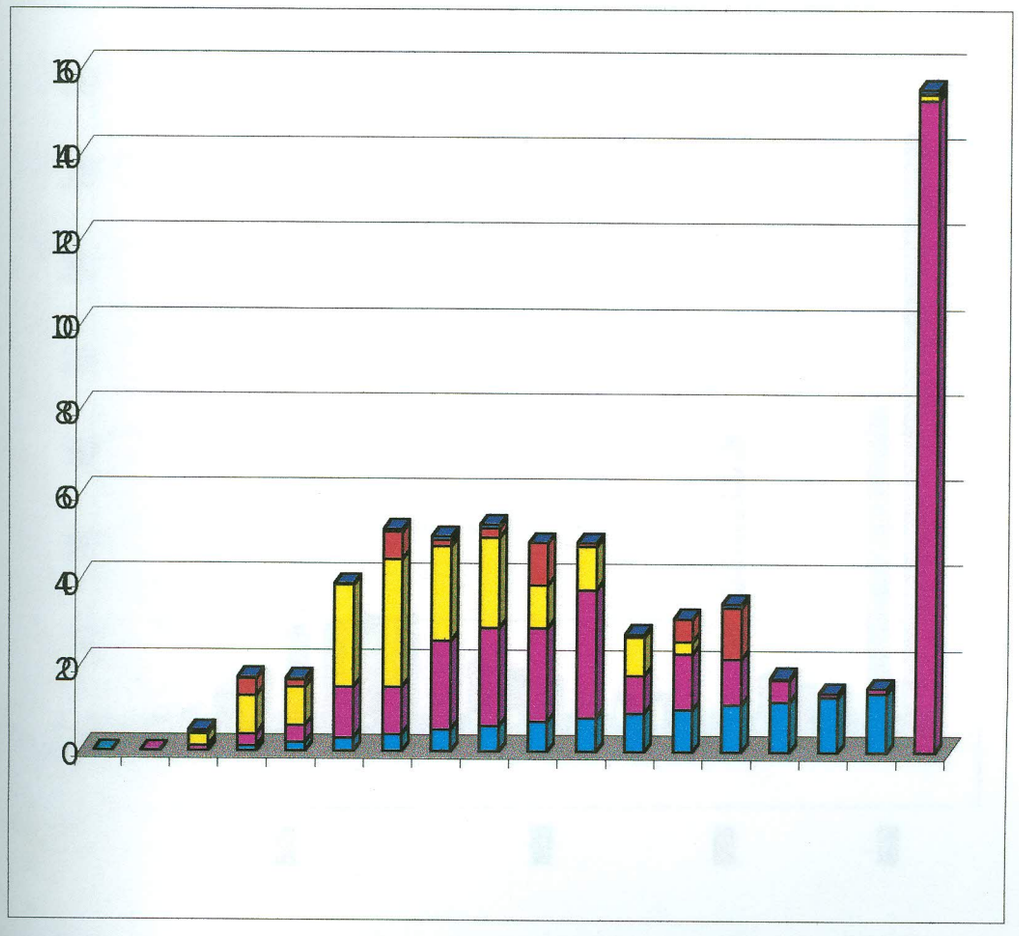
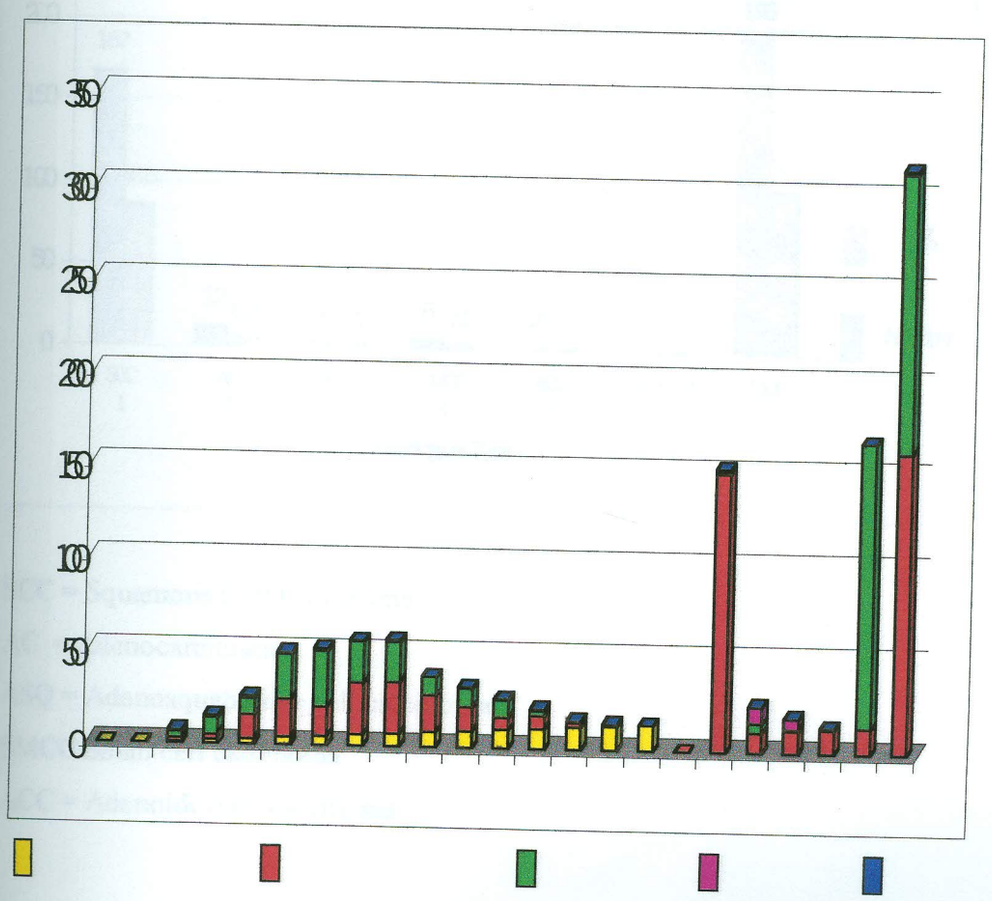


Figure 5. Frequency distribution of parity among patients with cervical cancer and controls



PA = parity

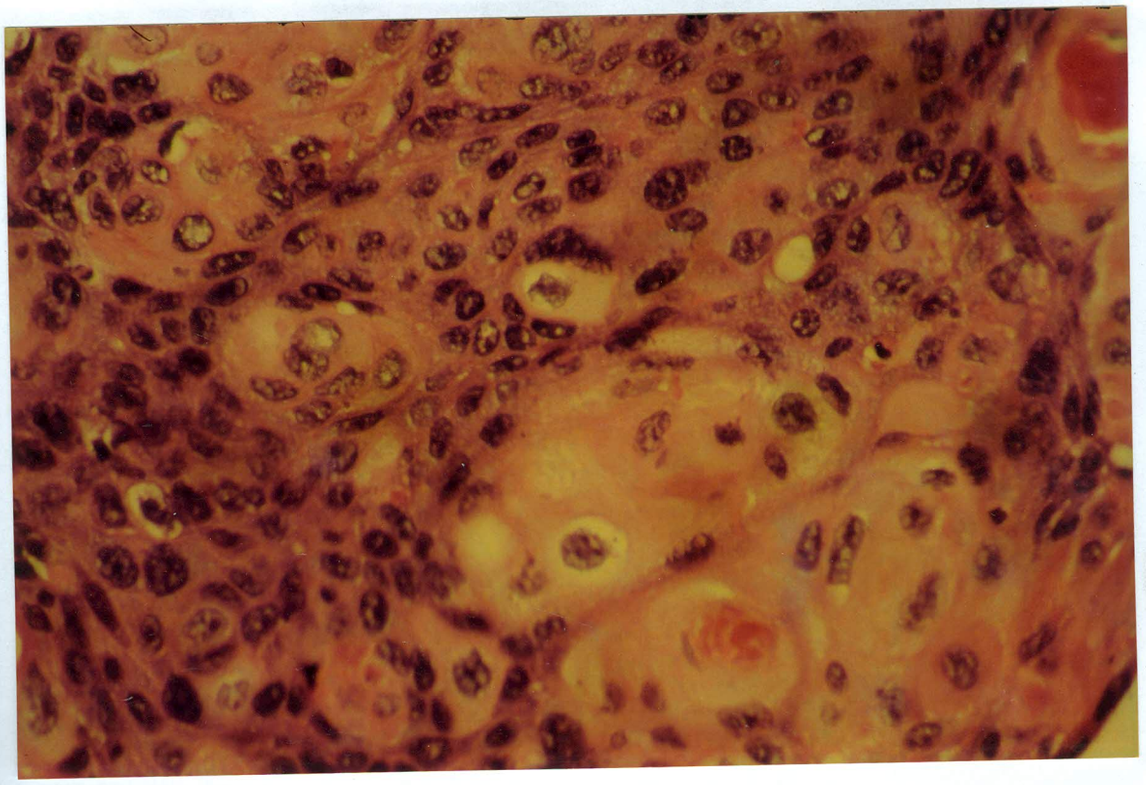


Figure 7(a): Section of the cervix showing a keratinizing, squamous cell carcinoma (H&E x 1000).

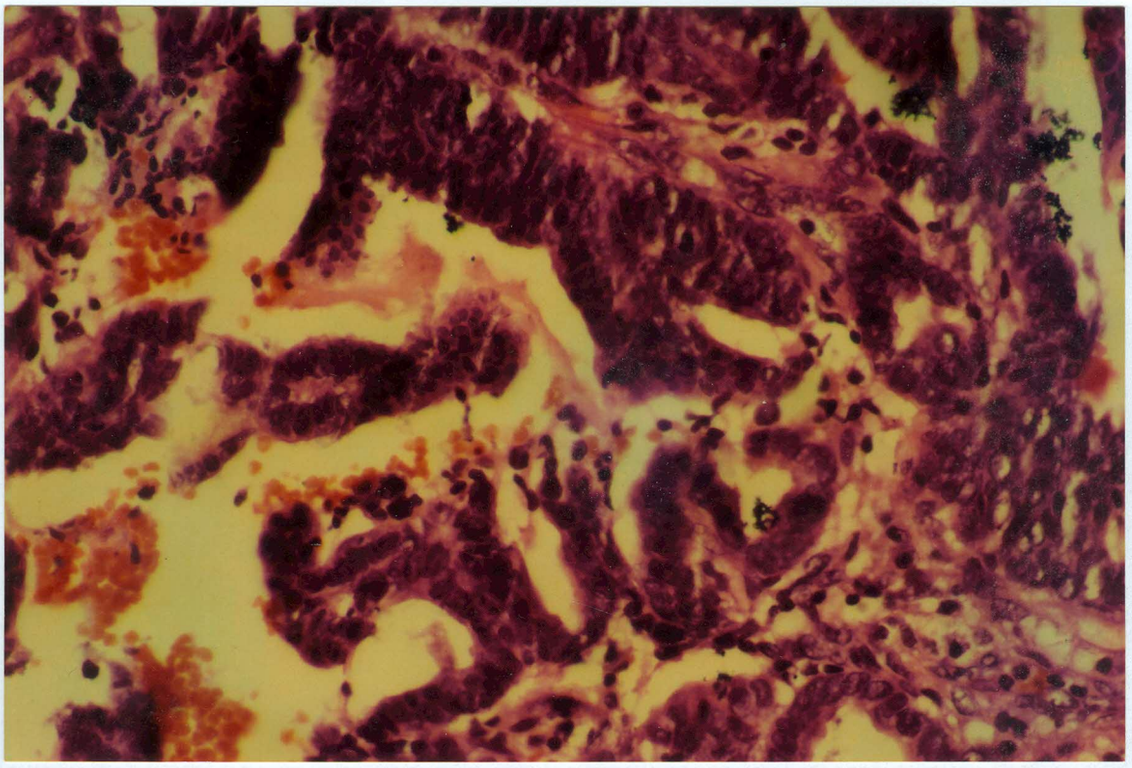


Figure 7(b): Section of the cervix showing adenocarcinoma (H&E x 1000)

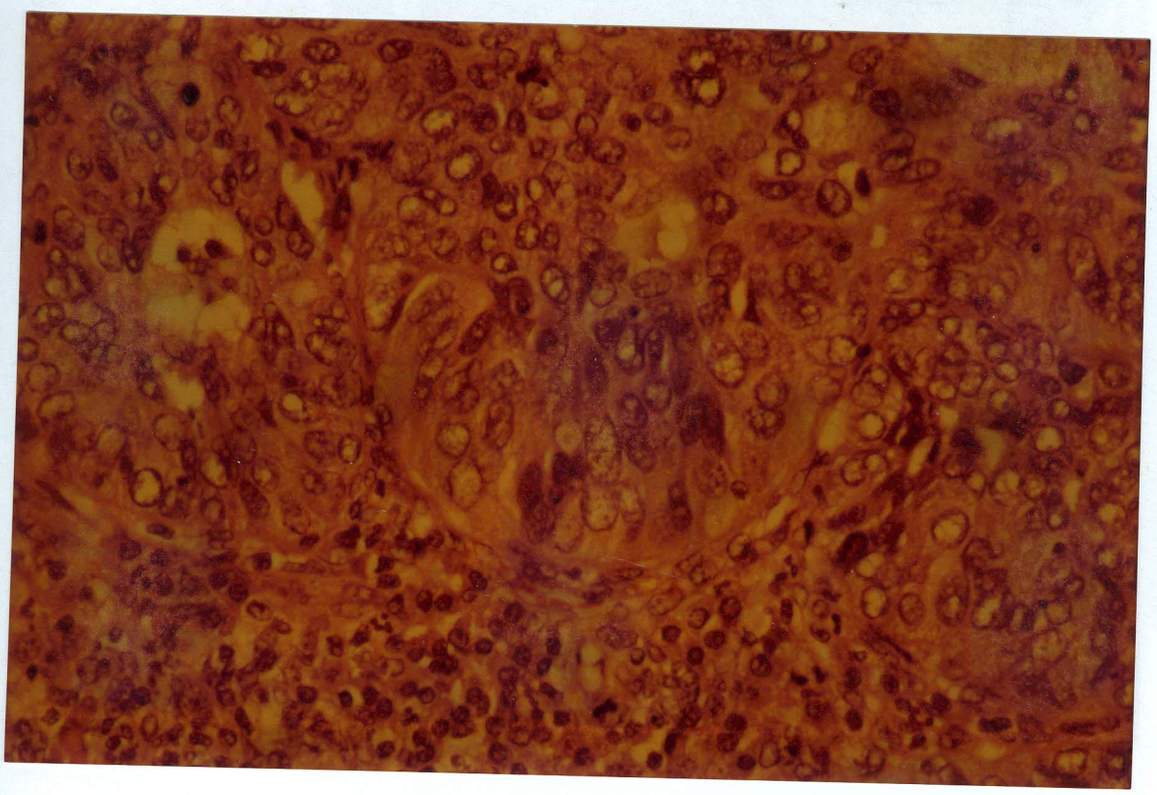


Figure7(c): Section of the cervix showing adenosquamous carcinoma (H&E x 1000)

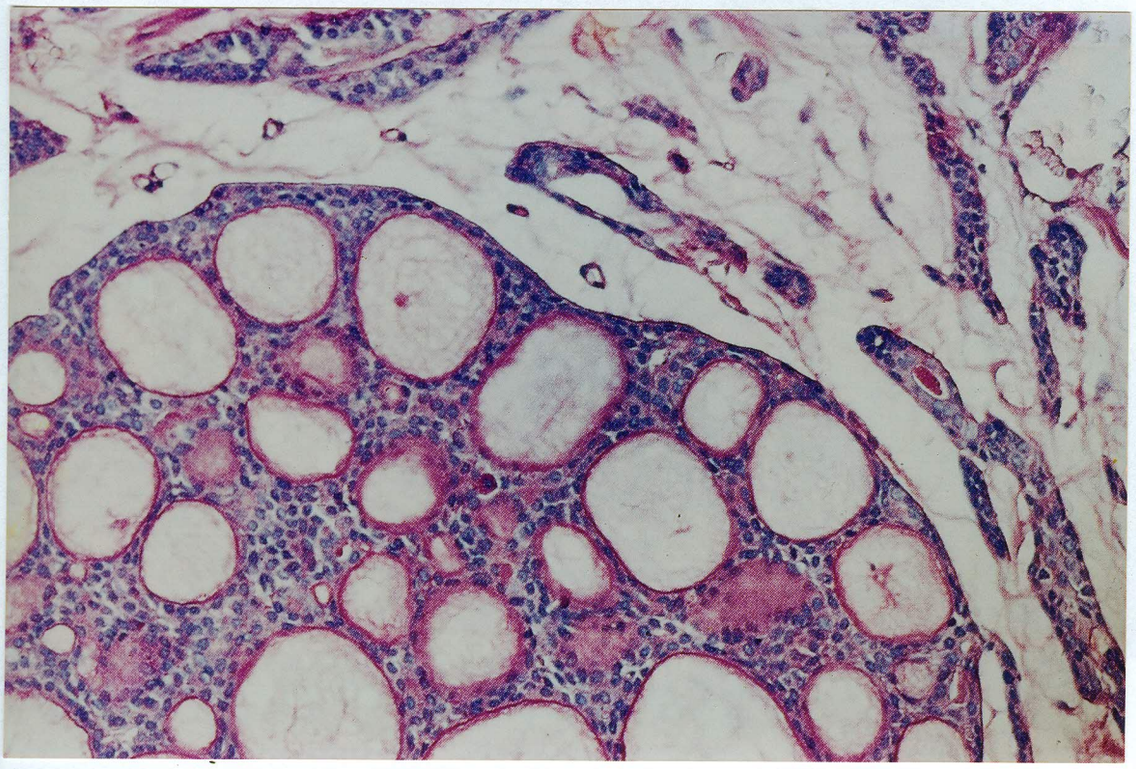


Figure 7(d): Section of the cervix showing adenoidcystic carcinoma (PAS x 1000).

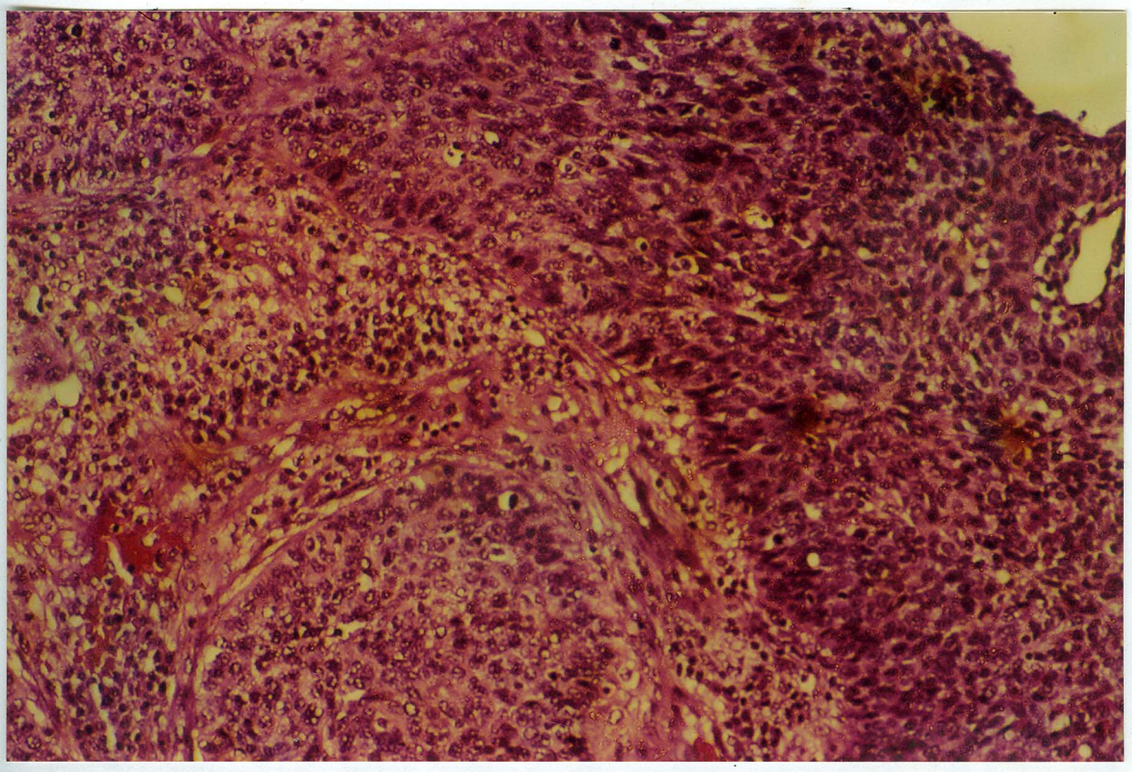


Figure 8(a): Section of the cervix. Undifferentiated small cell carcinoma (H&E x 1000)

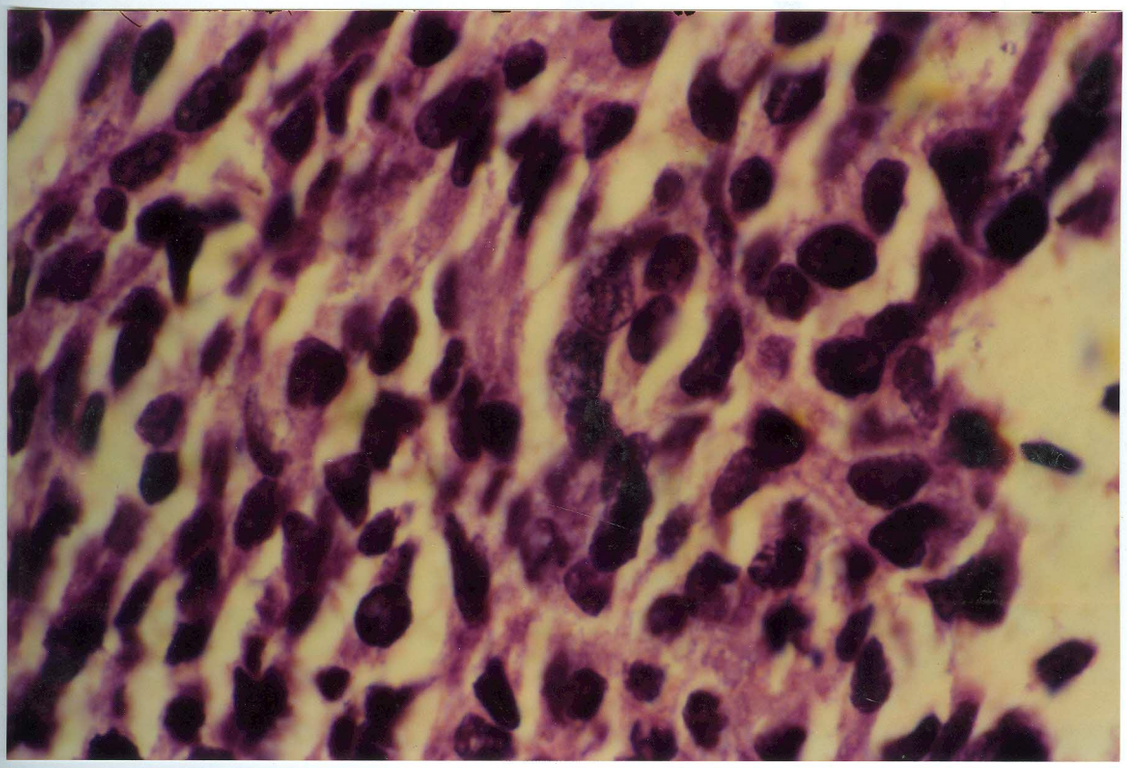


Figure 8(b): Section of the cervix showing carcinoid tumour (H&E x 1000)

Figure 9. Histological grading and subtypes of small cell carcinoma of the cervix

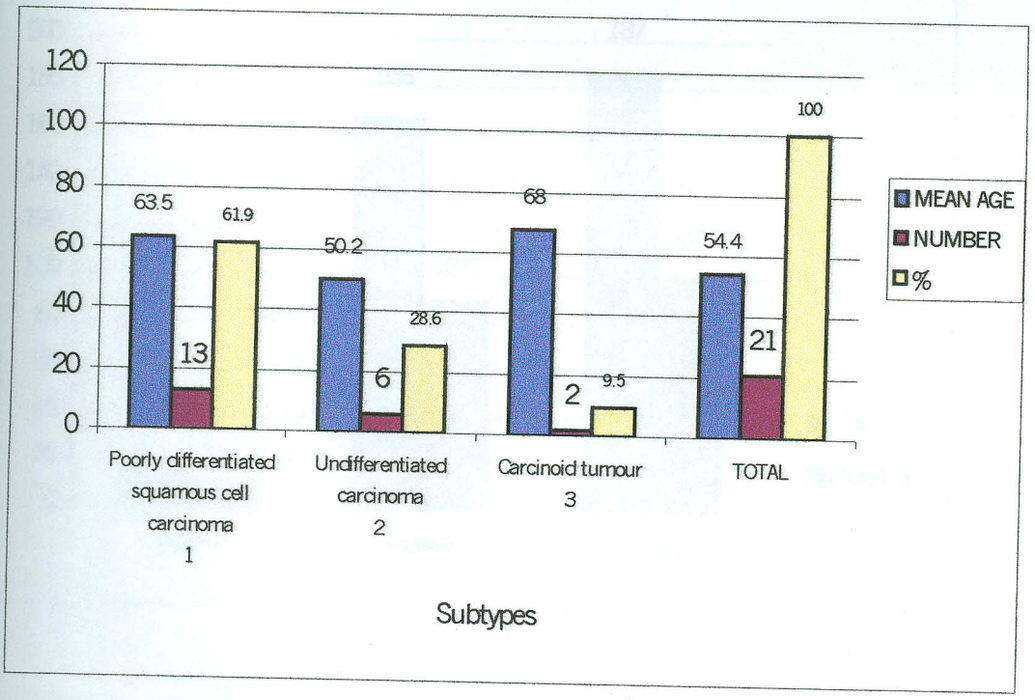
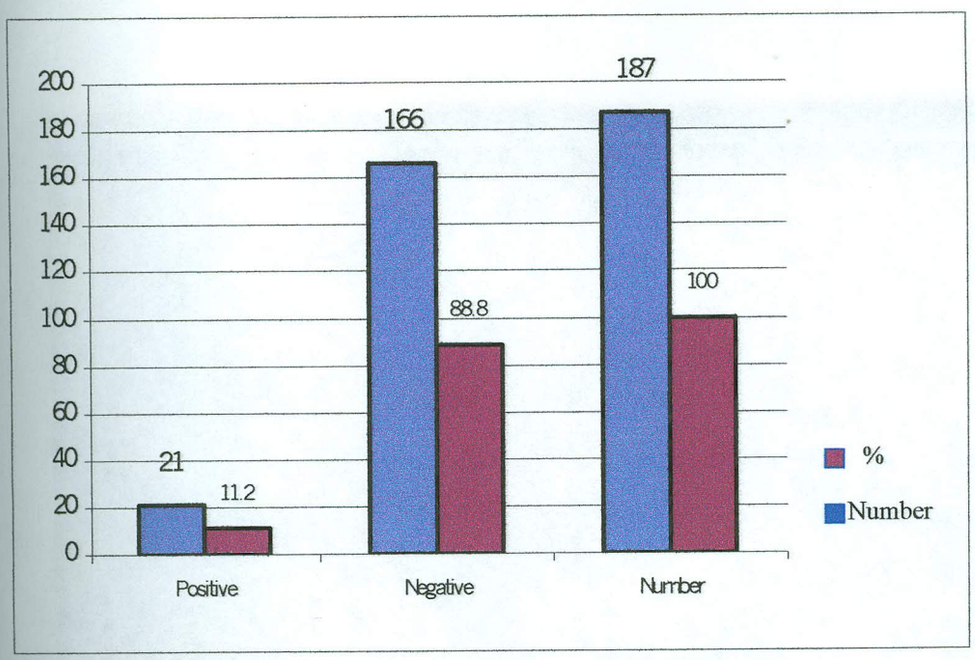


Figure 10. Serostatus of patients tested for HIV - 1



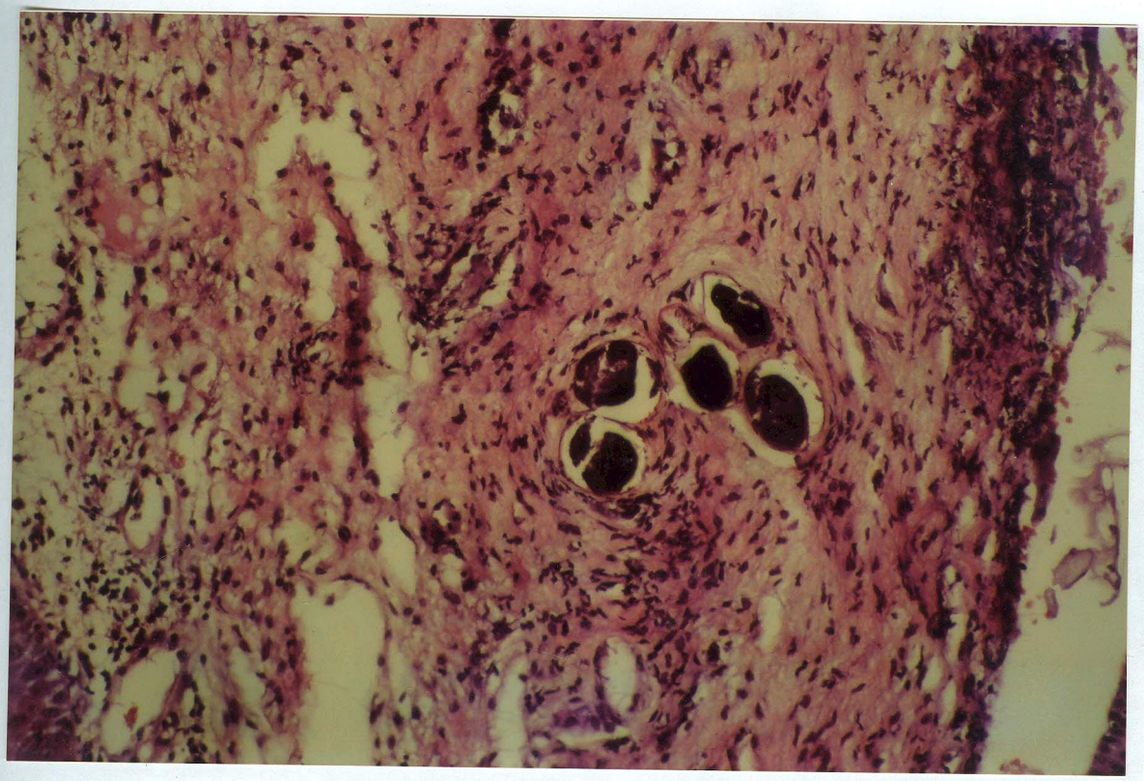


Figure 11(a):Section of the cervix showing schistosomiasis eggs (H&E) x 1000.

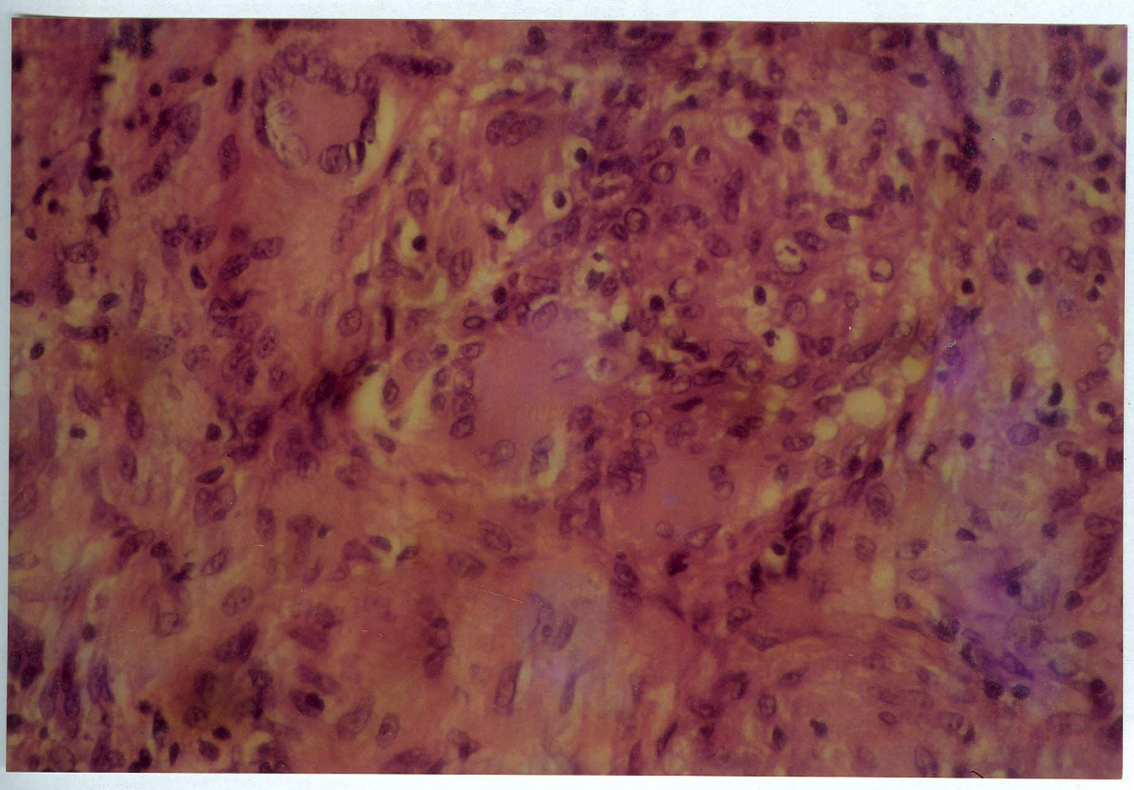


Figure 11(b): Section of the cervix showing tuberculous cervicitis. Note multinucleated giant cells, (H&E x 1000).

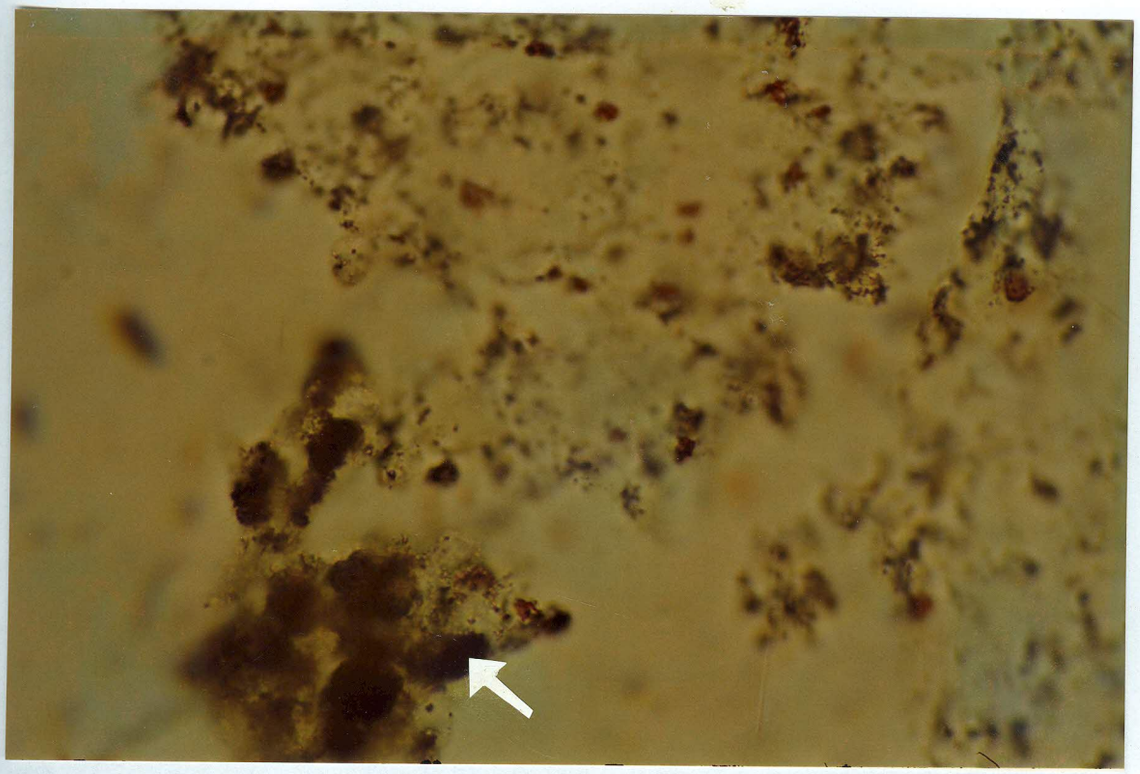


Figure 12(a): Section of the cervix showing small cell carcinoma (arrows) (Gilimerius x 1000).

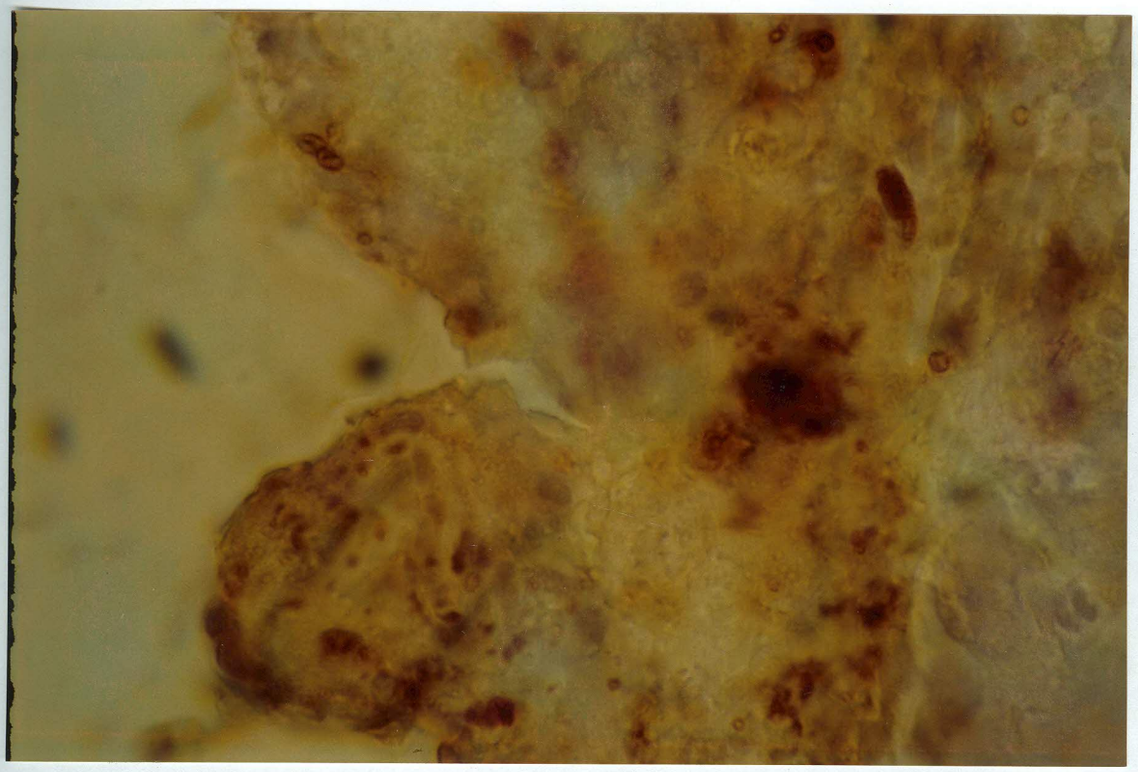


Figure 12(b): Undifferentiated small cell carcinoma of cervix immunoperoxidase staining for neuron specific enolase (NSE) x1000

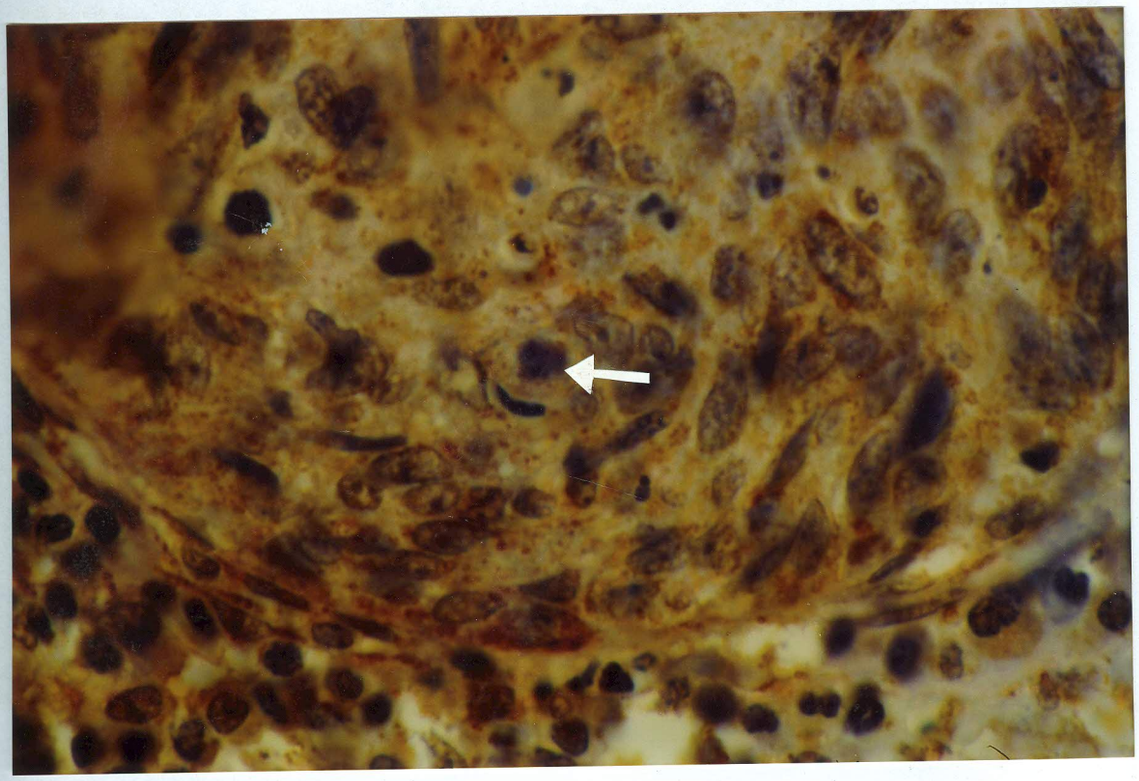


Figure 13: Squamous cell carcinoma of cervix showing immunoperoxidase staining for HPV – L1 antigen (arrow) (Anti-HPV – x 1000).

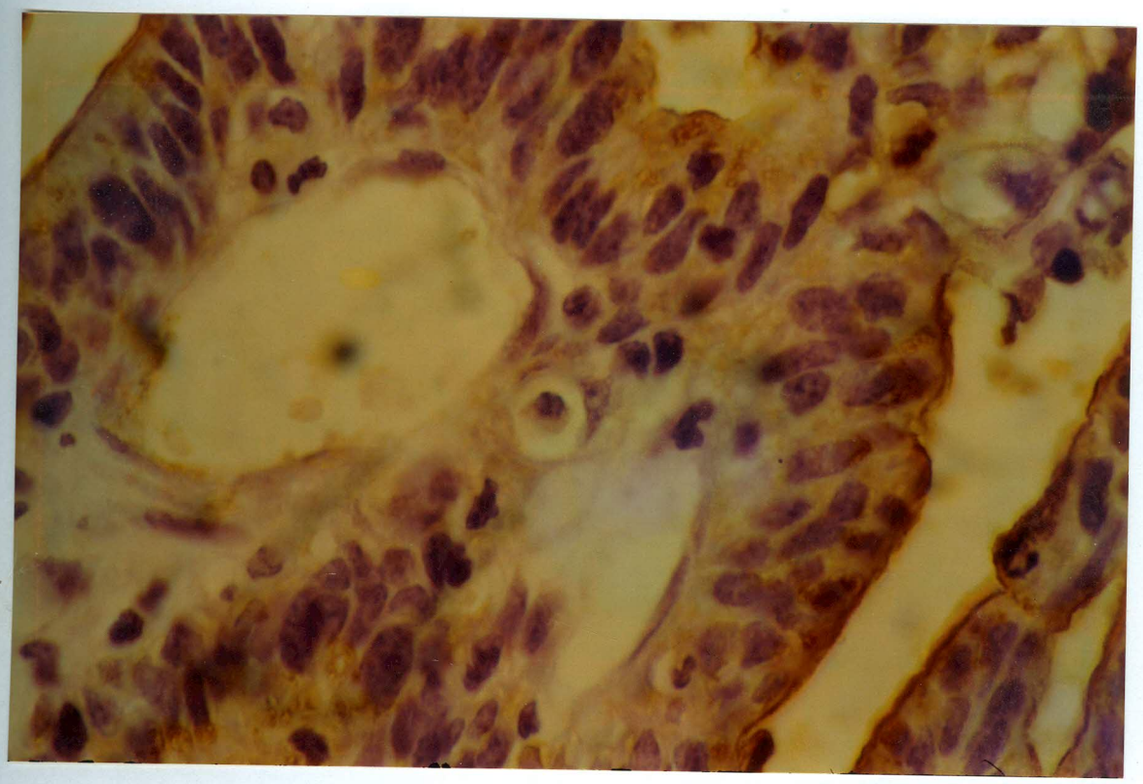


Figure 14: Cervical cancer immunostained with epithelial membrane antigen (EMA). Note the brown reaction product (x 1000).

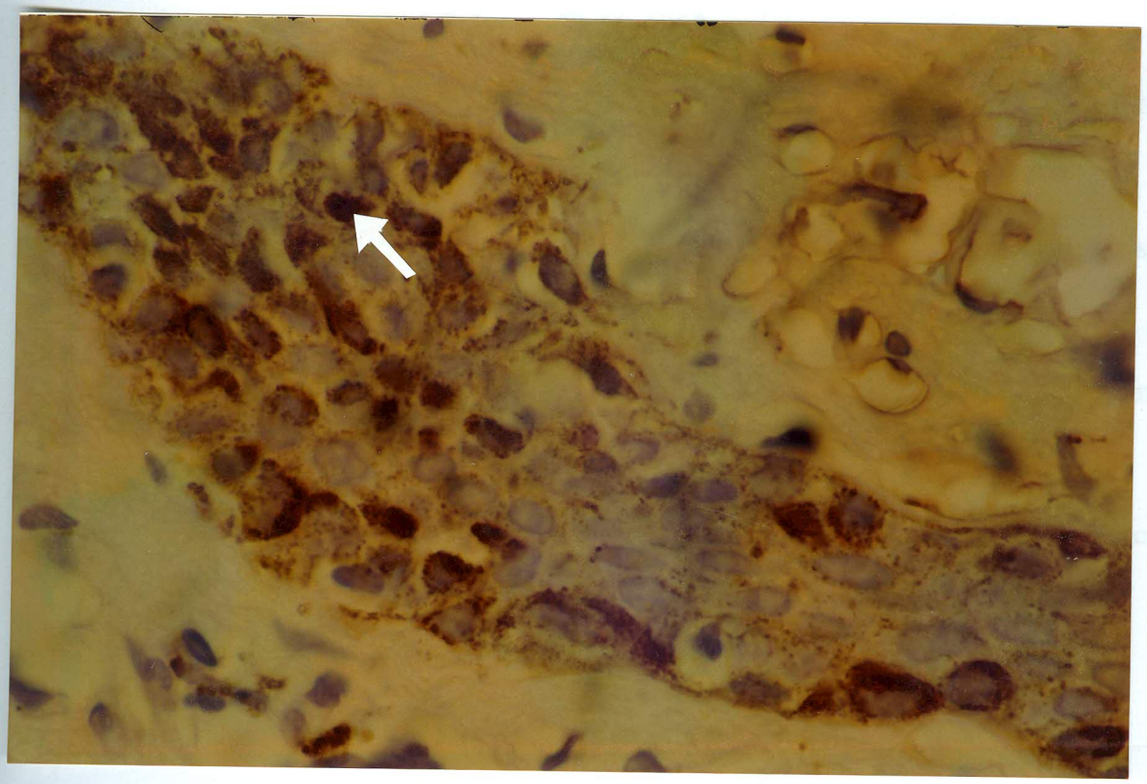


Figure 15: Squamous cell carcinoma, small cell type immunostained for Ki-67 antigen. Note black brown reaction product within the nucleus (arrows) (x 1000)

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