# Scholars Journal of Applied Medical Sciences (SJAMS) *Abbreviated Key Title: Sch. J. App. Med. Sci.* ©Scholars Academic and Scientific Publisher A Unit of Scholars Academic and Scientific Society, India www.saspublishers.com

ISSN 2320-6691 (Online) ISSN 2347-954X (Print)

Pathology

# The Relative Frequency and Patterns of Solid Malignant Tumors in Tikur Anbessa Specialized Teaching Hospital: A 10 Year Histopathology Review

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# **Driginal Research Article**

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**Article History** *Received:* 29.12.2017 *Accepted:* 15.01.2018 *Published:* 30.01.2018

**DOI:** 10.36347/sjams.2018.v06i01.057



Abstract: A ten year retrospective study was carried out consecutively on all histologically diagnosed solid malignant tumors to determine the relative frequency, and patterns of malignant solid tumors in the department of Pathology, Tikur Anbessa Specialized Teaching Hospital between 1st January 2003 and 31st December 2012 with a view of providing information on cancer burden. Pathology reports of all patients diagnosed with solid malignant tumors within the study period were retrieved and assessed with respect to age, gender, site, and histology types. Data were entered and analyzed using Statistical Package for Social Sciences Statistics 21.0. A total of 15,685 patients were histopathologically diagnosed with various solid malignant tumors. Most cancers were recorded among females accounting for (71.4%) of the total cases. The top ten frequently diagnosed cancers when both sexes considered together were cervical cancers in females accounting for (34.66%) followed by female breast (10.10%), lymphomas (7.92%), melanoma and non melanoma skin cancers ( 6.82%), soft tissue sarcomas (5.98%), colorectal (5.11%). esophagus (3.08%), secondary carcinomas in the lymph nodes (2.98%), eye and adnexa (2.93%), and bladder (2.98%). This study revealed cancers of the cervix, breast and lymphomas combined account for 52.6% of the total cases.

**Keywords:** Solid malignant tumors, Frequency, Patterns, Histopathology diagnosis, Ethiopia.

# INTRODUCTION

Globally, cancer is a major cause of mortality and morbidity. The burden of cancer will continue to shift to low income countries due to growth and aging of the population, and increasing prevalence of known risk factors such as smoking, alcohol consumption, over weight/obesity, physical inactivity, multiple and promiscuous sexual contacts, low intake of fresh fruits and vegetables, reproductive changes related to economic development and urbanization, and increased exposure to environmental carcinogens [1].

According to the report of International Agency for Research on Cancer (IARC), about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide, of which 57% of the cases and 65% of the deaths occurred in the economically developing world. Estimation of prevalence from the same data shows that there were 32.6 million people over the age of 15, who had had a cancer diagnosed in the previous five years [2]. Lung cancer is the leading cause of cancer death among males in both more and less developed countries. Other leading causes of cancer death in more developed countries include colorectal cancer in both sexes and prostate cancer among males [3]. In less developed countries, breast cancer remains

the leading cause of cancer death among females whereas highest cancer mortality in males is due to liver and stomach cancers. In some other less developed countries, cervical cancer remains the leading causes of cancer death among females [4]. Overall cancer incidence rates in developing world are half those seen in the developed world in both sexes. The overall cancer mortality rates are generally similar, and cancer survival tends to be lower in developing countries because of advanced stage at diagnosis and limited access to effective and efficient treatment [5]. By 2020, the total number of new cancer cases is expected to increase by 29% in developed countries whereas in developing countries an increase of 73% is expected.

This is largely as a result of aging, urbanization and change in dietary habits [2].Projections based on GLOBOCAN 2012 estimates a substantial increase to 19.3 million new cancer cases per year by 2025 due to growth and aging of the global population. As a result, GLOBOCAN 2012 highlights that priority should be given to cancer prevention and control measures for breast and cervical cancers worldwide [6]. Reliable high quality data on cancer in low income countries, especially Sub-Saharan countries, is a challenge for most countries. In this region, only 1% of the population is covered by population based cancer registries using data sets included in the latest volume (IX) for the Cancer Incidence in Five Continents (CI5) series of the IARC 12 [7]. Ethiopia, as one of the low countries in Sub-Saharan income Africa. is experiencing a double burden of communicable and non-communicable diseases including cancer. It is the second most populous country in Africa, next to Nigeria, with a population of over 100 million by 2017 and a population growth rate of 2.88% by estimates of 2016 [2]. There exists no national population based cancer registry in Ethiopia but the first population based cancer registry for Addis Ababa City was established in September 2011 at Radiotherapy Center in Tikur Anbessa Specialized Hospital. This has been the first type in the country with facilities for the treatment of cancers, since then information was collected to provide population based cancer incidence data, and for planning and monitoring cancer control activities in the country. The GLOBOCAN 2012 country specific databases indicate that cancer accounts 5.8% of the total national mortality in Ethiopia. The annual cancer incidence has been observed to be 60, 690 cases. The annual mortality has been over 44, 000, and the number of 5-year prevalent cancer cases in adult population for both sexes was 129,962 cases and the three most frequent cancers were breast cancer accounting for 30.2% followed by cervical cancer 13.4% and colorectal 5.7% [2].

Recognizing the rapidly increasing burden of cancer in Ethiopia, the Federal Ministry of Health has launched the first National Cancer Control Plan for the year 2016-2020 with the aim of reducing cancer incidence and mortality by 15% by 2020 through the control of risk factors associated with the development of cancer and promoting primary cancer prevention, surveillance, research, early diagnosis and improved cancer diagnosis and treatment [8]. The true magnitude of the cancer burden is reliably unknown in Ethiopia, largely because of nonexistence of population based cancer registry except for the newly established Addis Ababa City cancer registry. Hence, this hospital based retrospective histopathology review of relative frequency, patterns, age, gender, histological types, and frequency over time of malignant solid tumors was carried out in the country's largest teaching and referral University hospital providing diagnostic and treatment services for cancer patients from urban and rural areas of the entire country. This study was carried out with a view of providing large scale pathology based cancer base line data as to the burden of the disease and, potentially of interest to policy makers, clinicians, public health professionals, and cancer advocacy groups.

# METHODS

This was a retrospective descriptive study based on histopathology reports of all solid malignant tumors consecutively reviewed in the Department of Pathology, Tikur Anbessa Specialized Teaching Hospital from 1<sup>st</sup> January 2003 and 31 December 2012. The global objective of this study was to provide comprehensive cancer statistics to increase awareness on the nature and magnitude of the problem among policy makers, the general public, cancer advocacy groups and health professionals. Tikur Anbessa Specialized Hospital is the largest teaching and referral hospital in Ethiopia receiving Pathology specimens from all over the country. It provides a large scale pathology based data seemingly identified as a mini population based cancer data. The data base of all patients with histopathology diagnosis of solid malignant tumors were retrieved and entered into a study proforma. Data were organized by 5 year age group, gender, trends over time and all neoplasm were classified and coded by anatomical sites, histological types and behavior using Chapter 11 Neoplasm, International Statistical Classification of Diseases and Related Health Problems 10 Revision ( ICD-10)-WHO Version for; 2016. Data was entered into excel Microsoft spread sheet and was exported into SPSS version 21 for windows. Results are presented in simple percentages, tables, figures and charts. Approval for the use of histopathology reports archives for the purpose of publication was obtained from the Department of Pathology ethical clearance committee.

# RESULTS

Within 10 years-time (from 2003 to 2012), a total of 15,685 patients were histopathologically diagnosed for various forms of solid malignant tumors, of which 71.6% were females. The overall age distribution of patients with solid malignant tumors ranged from 1 year to 98 years, with a mean age of 45.1  $\pm$  15.9 years. The mean ages of females and males at the time of diagnosis were  $45.34 \pm 14.3$  and  $44.4 \pm 19.3$  years, respectively. Majority were in the age group between 35 and 54 (Table-1).

Table-1: General characteristic of cancer patients by age and sex		
Characteristics	Number	Perent
Sex		
Male	4465	28.5
Female	11220	71.5
Age group		
< 5 yr	184	1.2
5-9 yrs	203	1.3
10-14 yrs	220	1.4
15-19 yrs	350	2.2
20-24 yrs	563	3.6
25-29 yrs	934	6.0
30-34 yrs	1165	7.4
35-39 yrs	1670	10.6
40-44 yrs	1709	10.9
45-49 yrs	1970	12.6
50-54 yrs	2052	13.1
55-59 yrs	1278	8.1
60-64 yrs	1513	9.6
65-69 yrs	827	5.3
70-74 yrs	604	3.9
75+ yrs	443	2.8
Mean age + SD		
Male	44.4 <u>+</u> 19.3	
Female	45.3 <u>+</u> 14.3	
Overall	45.1 <u>+</u> 15.9	

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Fig-1: Age pyramid of patients with solid malignant tumors diagnosed at Tikur Anbessa Specialized Teaching Hospital from 2003 to 2012, Addis Ababa Ethiopia

The population pyramid of patients diagnosed with all types of solid tumors depicts increment in

proportion of cancer at lower age (between15-19) in females. The proportion of female cancer patients has

been progressively rising with increase in age, reaching the pick at an age between 45-49 years. In males, the general rise in magnitude of cancer burden takes place at the same age as females but with gradual increment reaching the pick at older age than in females (between 60-64 years) (Fig-1). The overall trend of malignant solid tumors reviewed at Tikur Anbessa Specialized Hospital indicated an annual average number of 1567 cases, ranging between 1300 per year in 2003 to 1885 cancer cases in 2012 (Fig-2).



Fig-2: Trend of cancer cases diagnosed at Tikur Anbessa Specialized Teaching Hospital from 2003 to 2012, Addis Ababa, Ethiopia

The pattern of the top ten solid malignant tumors diagnosed in this hospital between 2003 and 2012 are illustrated in Fig-3. Accordingly, malignant neoplasm of cervical uteri was almost three times more common than all other cancer cases combined and the most frequently diagnosed cancer in Tikur Anbessa Specialized Hospital. The current study indicated that malignant neoplasm of the breast is the second commonest solid malignant tumor followed by neoplasm of lymphoid tissues. Other malignant neoplasms, like malignant neoplasm of the skin, soft tissue, the colorectum, and esophagus are still common types of malignancies observed among the top ten malignancies in the study group. Solid tumors of the eye and adnexa, the bladder and secondary neoplasms of lymph nodes are observed at the bottom of the topten solid malignant tumor lists (Fig-3).





Fig-3: The ranking of the 10 most common cancers diagnosed at Tikur Anbessa Specialized Teaching Hospital from 2003 to 2012, Addis Ababa, Ethiopia

For the sake of simplicity and explanation, the trend of the top five solid malignant tumors diagnosed in Tikur Anbessa Specialized Teaching Hospital between 2003 to 2012 are illustrated in Fig-4. Interestingly, solid malignant tumors of cervix uteri was

the commonest malignant neoplasm diagnosed in the women population, and its occurrence ranged between 447 cases in 2003 and 639 women in 2009. The pattern of breast cancer indicated sharp increment from 93 cases in 2004 to 184 cases in 2007.





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Other common malignant neoplasms observed in this study were malignant neoplasm of soft tissues and the skin (Fig-5).

Pattern of solid malignant tumors among men population in this study was complex. Malignant neoplasm of lymphoid tissues was the dominant malignancy in all the study years, with an occurrence ranging between 53 cases in 2003 to 96 in 2009. The second common type of solid malignant tumor in men was malignant neoplasm of the the skin, and its occurrence ranged between 31 cases in 2003 and 86 cases in 2009. Other malignant neoplasms observed in men include malignant neoplasms of the colon and rectum, the soft-tissues and neoplasms of the bladder (Fig-6).



Fig-5: Trend of the top five solid malignant tumors in women population diagnosed at Tikur Anbessa Specialized Teaching Hospital from 2003 to 2012, Addis Ababa, Ethiopia



Fig-6: Trend of the top five solid malignant tumors diagnosed among men at Tikur Anbessa Specialized Teaching Hospital from 2003 to 2012, Addis Ababa, Ethiopia

The common characteristics of malignant solid neoplasms among children of pediatrics age are illustrated in Fig-7. Solid tumors of the eye and adnexa were the dominant malignancies in children, with an average occurrence of 14 cases per year (ranging between a minimum of 3 cases in 2008 to 23 cases in 2010). Second to the eye and adnexa in children was malignant neoplasm of lymphoid tissues that ranged between 8 cases in 2004 to 21 in 2007.



Fig-7: Trend of the top five solid malignant tumors diagnosed among pediatrics age groups at Tikur Anbessa Specialized Teaching Hospital from 2003 to2012, Addis Ababa, Ethiopia.

Other common solid tumors in children were malignant neoplasms of soft-tissues, bone and articular cartilage and secondary malignant neoplasms of lymph nodes (Fig-7).

#### DISCUSSION

Non communicable diseases including cancers contribute substantially to the burden of disease and to the demands on the health sector in the Sub-Saharan Africa, and will emerge as the main public health problem in few years later than any other region [9, 10]. In our study, a total of 15,685 patients (4465 males, and 11,220 females) were histopathologically diagnosed with various solid malignant tumors in the department of Pathology, Tikur Anbessa Specialized Referral and Teaching Hospital from January 2003 through December 2012 inclusive. Similar to previous studies from Ethiopia and other African countries, the majority of cancer cases were recorded among females, accounting for 71.4% of all cases [11-13]. In this study, the predominant age group in prevalence was between 45 and 54, accounting for 25.64% of all cases. This is in accordance with other studies from other Africa countries and low and middle income Asian countries [11-14]. A total of 607 childhood cancers of both sexes comprising 3.87% of all malignancies were recorded during the study period. This magnitude is excluding non-solid malignant tumors such as leukemia, one of the common cancer types in children. This indicates that solid pediatric tumors per se were comparable with the total childhood malignancies reported from middle income countries, where the incidence of pediatric malignancies varied from 4.1-12.6% [15-17]. The distribution of cancer in Ethiopia in this study is similar to that in other Sub-Sahara African countries with few exceptions. The top ten most frequently diagnosed solid malignant tumors comprise 82.38% of the total cancer cases. Majority of the cancer cases were reported from females of productive ages, indicating the seriousness of the problem. Within female cancers and over all cancer morbidity, cervical cancer was the most frequently diagnosed cancer, accounting for 34.66%, followed by breast cancer (10.10%), and Lymphoma (7.92%). These three cancer types combined constitute 52.6% of the entire cancer burden in the study area. Melanoma and non melanoma skin cancers comprise 6.82% followed by soft tissue sarcomas (5.98%). Colorectal cancers (5.11%), esophageal tumors (3.08%), secondary undifferentiated carcinomas in the lymph nodes (2.98%), tumors of the eye and adnexa (2.93%), and bladder (2.8%) are recorded in the top-ten cancer lists, respectively. A previous study conducted using hospital cancer registry in Tikur Anbessa Hospital Oncology center from 1998-2010 showed similar findings where cervical cancer was the most frequent cancer followed by breast cancer [11]. In line with our findings, Sitas et al., in their report of the major cancer types in Sub Sahara African countries pointed out that

cancers of the cervix (25.4%) and breast (17.4%) were the leading cancers in females, whereas in males contrary to our findings Kaposi sarcoma (15.9%), liver (13.3%), and prostate (10.7%) cancers were the top three forms of tumors in their report [18]. Despite the AIDS epidemic in Ethiopia there has not been obvious increase in the frequency of Kaposi sarcoma. Similarly, hepatitis B and C infections are very common and there exists high environmental exposure to afla toxin in the population, but liver cancer is rare in this report. Our study also showed a low frequency of prostate cancer, which is of course a disease of elderly males. The lower life expectancy in Ethiopia and lack of post mortem may significantly contribute to the low relative frequency in this study. On the whole, in Ethiopia most of the patients first prefer to go to the local healers and eventually by the time they seek medical services, the cancers are detected at advanced stage. Thus, the diagnosis is most often based on clinical, imaging, or cytological, and invasive procedures such as tissue biopsy is rarely performed. Furthermore, in low resource countries like Ethiopia owing to financial constraints a segment of patients with cancers may not at all get access to modern diagnostic and treatment facilities but rather left behind without any care. Hence, this shows the discrepancy observed and point out that this hospital pathology based cancer data may not truly reflect the actual cancer profile for the entire country. In our data, like in many countries in Sub-Sahara African countries based on urban population cancer registries, cancer of the cervix is the leading cancer in women. This is due to lack of organized national screening programs for cervical pre-invasive lesions [11, 19-27]. Realizing the burden of cancer in Ethiopia, the Federal Ministry of Health developed and launched the National Cancer Control Plan for 2016-2020 to introduce as part of control of cervical cancer, a population based cancer screening using VIA (Visual Inspection after Acetic acid impregnation of the cervix) at all health levels for all women aged 30-49 every 5 years. The plan also includes rapid screening of large number of women by HPV DNA testing, and avail cryotherapy treatment in a single visit approach, and develop effective referral system for advanced treatment [8]. In our study, next to cancer of the cervix, breast cancer is the second most frequently diagnosed tumor in women in line with some other countries in Sub-Saharan Africa and in the world [11, 18, 28]. Low rates of breast cancer have been reported from population based cancer registry data in Harare, Zimbabwe [24] for the period 1990-1992. However, breast cancer ranks third in the Gambia data collected over a 10 -year period (1988-1997) using the Gambian National Cancer Registry [19]. Breast cancer is the leading cancer in women from hospital based cancer data (2000-2002) from one of the developing countries in Asia-Pakistan [14]. Breast cancer on the whole has now overtaken cervical cancer as the most commonly diagnosed cancer in women in several Sub-Saharan African countries [29]. Concerning breast cancer, reproductive factors may not be considered as

risk factors in Ethiopia as early marriages, early age at first pregnancy, multiple births, and prolonged breast feeding are common social norms, besides obesity, early menarche, hormonal exposure and late menopause are not considered significant. Other risk factors such as dietary, aging and genetic susceptibility may be operative but open for further investigation. Breast cancer control and prevention strategies have also been included in the Ethiopian National Cancer Control Plan document [8]. In our study, lymphomas (more than 85% represent non-Hodgkin's lymphoma) in both sexes and all ages constitute 7.92% of all cancer increase associated with cases. an Human Immunodeficiency virus infection/AIDS epidemic. Similar cancer profile study done in Larkana, Pakistan (2000-2002) showed lymphomas as the leading malignancy in males, and the third most common cancer in females [14]. According to the major cancer types in Sub-Saharan Africa reported by Sitas et al., non Hodgkin's lymphoma ranks the fifth (5.8%) and the six (3.8%) top cancers in males and females, respectively [18]. Another population based Cancer Registry report from Butare, Rwanda (1991-1992) showed non –Hodgkin's lymphoma as the fifth (4.3%) most frequent cancer in men [20]. Non-Hodgkin's lymphoma was the third most frequent cancer in males from the Kampala Cancer Registry data in 1889-1991[21]. Furthermore, a population based Cancer Registry data (2009-2010) from Nigeria indicated that non-Hodgkin's lymphoma (4.8%) rank fifth in males [13]. Earlier study by Wabinga et al., revealed an increase in the incidence of non-Hodgkin's lymphoma with the emergence of the AIDS epidemic in Uganda in 1990s [30]. Most of these findings were in agreement with earlier reports from Ethiopia. Previous hospital based cancer registry study done in Ethiopia (1998-2010) revealed lymphoma as the fifth most common cancer in males [11]. Study from Cancer Registry in Abidjan, Ivory Coast for the period 1995-1997 showed non-Hodgkin's lymphoma comprising 10.5% in men and 7.3% in women, and rank third in both sexes as the most common cancers [31]. On the whole, non-Hodgkin's lymphoma is more common in developed areas but is low in Africa with the exception of Burkitt's lymphoma in children as was observed in our data [32]. The current study shows that melanoma and non-melanoma skin cancers rank fourth (6.82%) in both sexes and all ages. Similar study from Cancer Registry study (1991-1992) in Rwanda demonstrated nonmelanoma skin cancer as the most frequent cancer in males [20]. Cancer profile study in Larkana, Pakistan (2000-2002) showed skin cancer as the fourth most common cancer in females [14]. Studies from Nigeria-Ibadan (2009-2010) showed that non-melanoma skin cancer is the third and the fifth in men and females. respectively [33]. Our findings indicate that soft tissue sarcoma ranks fifth (5.98%) in both sexes and all ages. Previous reports from hospital based cancer registry data in Ethiopia (1998-2010) showed sarcoma as the second most frequent cancer but without mentioning

whether or not bone or soft part sarcomas [11]. In our data colorectal cancer (5.11%) rank sixth, cancer of the esophagus (3.08%)rank seventh, secondary undifferentiated carcinomas (2.98%) in the lymph nodes primary being from head and cancers rank eighth, cancers of eye and adnexa (2.93) rank ninth, and bladder cancer (2.8%) rank tenth for both sexes and all ages. Colorectal cancer is the third most common cancer worldwide after lung and breast cancers, and four fold higher in more developed compared with less developed regions of the world [28]. In our study, we observed colorectal cancer as the sixth most frequent cancer in both sexes and all ages and observed at lower ages than the usual global reports. This could be an indication that our population is shifting towards adopting westernized life style and abnormal life styles mostly associated with modernization. A high burden of esophageal cancer is reported in Eastern and Southern Africa [29], which is in line with our finding, even though modest increase was observed. Previous reports indicated that head and neck cancers are the leading among the top six cancers in males and most of these tumors present with metastatic tumors in the neck lymph nodes the primaries being subclinical, which is in keeping with our findings that this secondary cancers rank eighth. Globally, nasopharyngeal cancer rates are highest in South-Eastern Asia. Similarly, the rates of nasopharyngeal cancer are also increased in Africa [32]. Cancers of the eye and adnexa in both sexes and all ages rank ninth (2.93%) in our study. Adults of both sexes and all ages are predominant due to squamous cell carcinoma of the conjunctiva, possibly associated with an AIDS epidemic. The increase in cancer of eye and adnexa in adults both sexes, all ages are predominantly due to squamous cell carcinoma of the conjunctiva possibly associated with an AIDS epidemic. In children the increase is attributed to high frequency of retinoblastoma and lymphomas particularly Burkitt's lymphoma type. Using data obtained from the Cancer Registry of the University of Nigeria Teaching Hospital for the period 1999-2004 Ochen et al., reported the incidence of retinoblastoma as the less common tumor [34]. Retinoblastoma accounted for 23.1% in males, and 22.4 % in females of ocular malignancies characterized histologically from Karachi Cancer Registry in 5 years (1998-2002) [35].Retinoblastoma accounted for 1.1% of all malignancies analyzed in the Cancer Registry of University of Ibadan, Nigeria [36]. In this study, majority of bladder cancers were histologically urothelial papillary carcinomas, both invasive and non invasive and rank tenth (2.8%) in both sexes and all ages. This is not in agreement with a large series of bladder cancer cases reported in some parts of Africa proved to be squamous cell carcinomas caused by chronic infection with the parasite Schistosoma hematobium. Particularly, the Egyptian men have the highest bladder cancer rates worldwide [29].

# Limitations

First and foremost, this study is a hospital based histologically proven data on relative frequency and patterns of solid malignant tumors observed for the defined period of study. It is a well-established fact that information on cancers from the Cancer Registries or data bases of large Referral centers for cancer patients and Teaching Hospitals as our setting represent only a segment of population. Secondly, the proportion of cancer patients with access to health service is undoubtedly limited in resource constrained Sub-Sahara African countries like Ethiopia. If at all visit health institutions, patients appear at advanced stage when invasive procedures like tissue biopsy is rarely performed. Hence, under reporting is a possibility. Furthermore. besides morphologic histological diagnosis of tumors, access to ancillary modern such diagnostic pathology facilities as immunohistochemistry and molecular tests are not available making definitive diagnosis at times difficult.

# CONCLUSIONS

This study revealed cancers of the cervix, breast and lymphomas combined account for 52.6% of the total cases. Cancers of the cervix and breast alone combined constitute 44.76% of the total cases and 71.4% of the tumors occurred among females. As both the cervix and breast are the essential reproductive organs in females and most of these cancers occurred in productive ages of the population, our finding has strong psychosocial and political implications. As Tikur Anbessa Specialized Hospital is the only national cancer referral center for cancer patients and the department of Pathology receives pathology specimens almost from the entire country we do believe that this pathology based hospital study despite its limitations fairly reflects the magnitude of the cancer problem in the country. This study also provides the basis for evaluating priorities for cancer control and prevention measures for policy makers and further strengthen the rationale for the national population based screening procedures already underway to reduce and prevent the two most frequently occurring cancers- cervical and breast cancers.

## Abbreviations

- GLOBOCAN- Global Cancer Incidence, Mortality and Prevalence
- IARC-International Agency for Research on Cancer
- AIDS -Acquired Immunodeficiency Syndrome
- SPSS -Statistical Packages for Social Sciences

## Declarations

#### Ethics approval

Ethical clearance was obtained from Ethical review committee of the department of Pathology and access to histopathology data was granted from the head of the department of Pathology, College of Health Sciences, School of Medicine, Addis Ababa University.

# **Consent for publication**

Not applicable

#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## **Competing interests**

The authors declare that they have no competing interests

Funding

No specific funding was disclosed

## Authors' contributions

Conception and design TG, WL, ND, Collection and assembly of data TG, WL, ND Data analysis and interpretation TG, WL, ND Manuscript writing TG, WL, ND All authors (TG, WL, and ND) read and approved the final manuscript

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