**INTRODUCTION TO HISTOPATHOLOGY**

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**CANCER EPIDEMIOLOGY.**

**IN AFRICA (Nigeria):**

***Introduction***

Cancer is a public health problem worldwide affecting all categories of persons. It is the second common cause of death in developed countries and among the three leading causes of death in developing countries.

The epidemiology of cancer is the study of the factors affecting cancer, to infer possible trends and causes. The study of cancer epidemiology uses epidemiological methods to find the cause of cancer and to identify and develop improved treatments.

During the 65th World Health Assembly, member states of the WHO agreed to adopt a global target of a 25% reduction in premature mortality from the four major noncommunicable diseases (NCD) by the year 2025. This was in response to the growing burden of NCDs, in which, in 2011, cancer was estimated to be the leading global cause of death, ranking above 0/kiischemic heart disease, stroke, and lower respiratory tract infections (World Health Organization. Global Health Observatory (GHO), 2011).

Cancer is an increasing problem in Africa because of aging and growth of the population as well as increased prevalence of risk factors associated with economic transition (including smoking, alcohol, obesity, physical inactivity, and reproductive behaviors), and of certain infectious agents of importance in cancer etiology. According to United Nations population estimates (United Nations Population Division. World population prospects, the 2012 revision.), the population of Africa between 2010 and 2030 is projected to increase by 60% overall (from 1.03 billion to 1.63 billion) and by 90% for those 60 and older (from 55 million to 103 million), the age at which cancer most frequently occurs.

Despite this growing burden, cancer continues to receive a relatively low public health priority in Africa, largely because of limited resources and other pressing public health problems, including communicable diseases such as Acquired Immune Deficiency Syndrome (AIDS)/Human Immunodeficiency Virus (HIV) infection, malaria, and tuberculosis. Another factor may be a general lack of awareness among policy makers, the general public, and international private or public health agencies, concerning the magnitude of the current and future cancer burden on the continent and its economic impact.

WHO reported that about 24.6 million people live with cancer worldwide1 12.5% of all deaths are attributable to cancer and if the trend continues, it is estimated that by 2020, 16 million new cases will be diagnosed per annum out of which 70% will be in developing countries1 Parkin et al reported that in indigenous Africans, 650,000 people of estimated 965million are diagnosed of cancer annually and lifetime risk of dying from cancer in African women is 2 times higher than in developed countries.

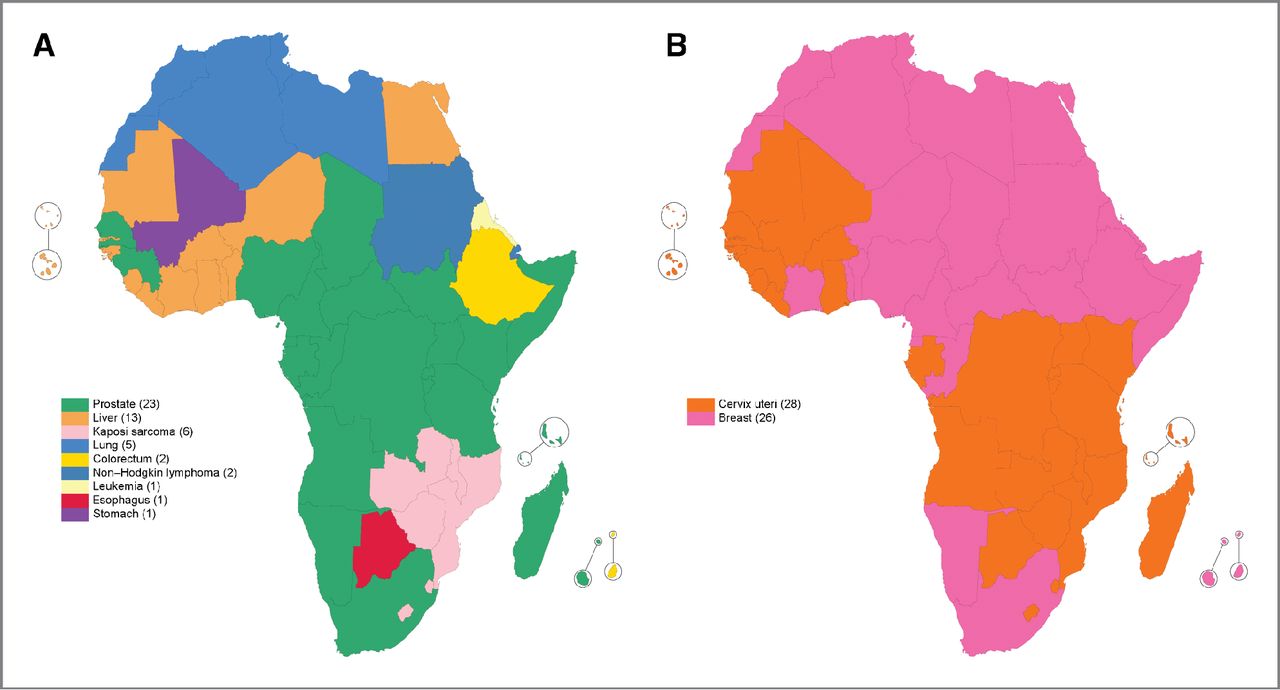


Fig 1. The sources of incidence data used for Globocan 2012 (A) and the method used to make the national estimate of incidence (B), by country.

***Data source and methods***

In seven countries (Algeria, Egypt, Libya, Malawi, Tunisia, Uganda, and Zimbabwe) there were local (“regional”) registries which cover less than 10% of the national population, but which were judged to be of sufficient quality for inclusion in the latest volume of “Cancer Incidence in Five Continents” (CI5; Forman, Bray, Brewster, *et al.,* 2013).

Observational epidemiological studies that show associations between risk factors and specific cancers mostly serve to generate hypotheses about potential interventions that could reduce cancer incidence or morbidity. Randomized controlled trials then test whether hypotheses generated by epidemiological studies and laboratory research result in reduced cancer incidence and mortality. In many cases, findings from observational epidemiological studies are not confirmed by randomized controlled trials.

***Cancer incidence data***

The earliest study from Nigeria was from the Ibadan Cancer Registry-1960-69(ICR); Edington & MacLean reported higher rates of cancer in females with age standardized rates (ASR) of 105.1 and 78 per 100,000 females and males respectively4,5. In 1998, 74.5 per 100,000 females and 63.9 for males was recorded from the same center4,6. In Zaria, 1976-78 data reported 1575 cases with 52% of cases in males and 48% in males; a latter study however showed more cancers in females than males8,9.

Current data (2001-2005) from Ibadan showed increasing incidence and the ASR for all cancers as 81.6 per 100,000 for males and 115.1 per 100,000 for females with 65.9% and 34.1% in females and males respectively7. From Kano, of 1001 cancers recorded for period 1995-2004, male cancers accounted for 50.3% and 49.7% in females10. Mandong et al recorded 1162 and 1657 cancer cases respectively for males and females for the period between 1995 and 2002 from the Cancer Registry in Jos University Teaching Hospital11,12. Report from University of Benin Teaching Hospital showed 2258 cases over a 20year period with female cancers predominating(64%) while that from Calabar showed a total of 588 cancers between 2004-2006 with 50.9% and 49.1% respectively for males and females13,14.

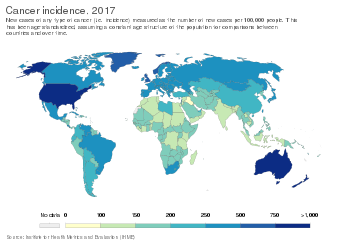


Fig 2. Cancer incidence in 2017.

The WHO estimated incidence of cancer from all sites in 2002 for Nigeria was 90.7 and 100.9 per 10,000 for males and females respectively while mortality rates were 72.2 and 76 respectively-Globocan15. This is comparable to 89.1 and 104.1/100,000 incidence for males and females and 72.2 and 79.6 crude mortality rates recorded for Ghana but much less than figures recorded for United Kingdom and USA. Generally, cancer incidence in Nigeria appears low compared to developed countries which may not truly reflect the burden. Like reports from other parts of the world, it is slightly higher in female.

**Table 1:** Estimate of cancer incidence, mortality and most common cancers in Nigeria as reported by GLOBOCAN, 2012.

|  |  |  |  |
| --- | --- | --- | --- |
| **Nigeria** | **Male** | **Female** | **Both sexes** |
| Population | 84,398,000 | 82,231,000 | 166,629,000 |
| Number of new cancer cases. | 37,400 | 64,700 | 102,000 |
| Age standardized rate (w) | 79.0 | 121.7 | 100.1 |
| Number of cancer death. | 30,900 | 40,600 | 71,600 |
| Age standardized rate (w) | 67.4 | 78.0 | 72.1 |
| 5-year prevalent cases, adult population. | 67,000 | 165,000 | 232,000 |
| Proportion (per 100,000). | 139.8 | 348.6 | 243.6 |

Nigeria recorded 102,079 cases of cancer, out of which 27,304 (26.7%) cases were for breast cancer, 14089 (13.8%) for cervix uteri, 12,047 (11.8%) for liver and 11,944 (11.7%) for prostate cancer as incidence (Figure 4) (Globocan, 2012). The age standardized incidence rates (ASR) for these common cancers; breast, cervix uteri, liver and prostate were 50.4, 29.0, 11.5, and 30.7 per 100,000 respectively. A 5-year prevalence study in Nigeria also showed almost the same trend. Breast cancer being the leading cases with 87,579 (37.7%), followed by cervix uteri 35,644 (15.4%), prostate cancer 31,062 (13.4%) and then liver 8,447 (3.7%).

***The six most common cancers in Nigeria in descending order of frequency are:***

Breast, cervix, prostate, colorectal, liver cancer and NHL.

* Breast cancer

Breast cancer is the most commonly diagnosed cancer in Africa, and in Sub-Saharan Africa, and is also the leading cause of death from cancer. It is a global disease of significant burden and its incidence continues to rise especially in the sub-Saharan Africa [Nggada, Yawe, *et al.,* 2008]. It was described as the most common cancer in women worldwide (Ihezue, Ugwu *et* *al.,* 1994). It accounted for 24.45% of all the cancer types. Huge differences have been observed in the behavior of the tumor, clinical manifestation, treatment response and prognosis across the various regions of the world especially between the developed and the developing world [15]. Apart from the island populations of Mauritius and Reunion, the highest rates are seen in Egypt, Algeria, Nigeria, and Republic of South Africa. Although the reasons for the increasing importance of breast cancer must be speculative, they most likely include increases in the prevalence of risk factors such as early menarche, late child bearing, having fewer children, obesity, and increased awareness and detection, which are associated with urbanization and economic development. There have been rapid increases in the incidence of breast cancer in Sub-Saharan Africa; rates of increase in the last 20 years were 3.6% per year in Kampala (Uganda) and 4.9% per year in the Black population of Harare (Zimbabwe; Nambooze, Amulen *et al.,* 2013). In North Africa, the increase in Central Tunisia was 2.5% annually in the last 15 years (Missaoui, Landolsi *et al.,* 2012).

It is the commonest female cancer and most common cancer in both sexes. Studies have indicated increase in the relative frequency ratio; moving from number 2 or 3 to the number one cancer in both sexes. This increase has been attributed to increase awareness and presentation for screening. Majority of breast cancers occur in pre-menopausal women with the peak age in the 5th decade.

Parity is 5.3-6 and age at first child is <20yrs. The most common histological type is invasive ductal carcinoma (not otherwise specified NOS) in 73-80% of cases (Jemal and Fedewa, 2012). About 80-85% still present in advance stage III with attendant poor outcome. In Nigerian studies, only 25-50% of the tumours are reported to be oestrogen/progesterone receptor positive, which is the basis for hormonal treatment23-24. It is common practice to give anti-oestrogen blindly in Nigeria without recourse to ER/PR status, this is reported to be at the risk of complication such as endometrial carcinoma, which has been reported in Nigeria (Banjo *et al.,*).

The risk factors for breast cancer-

female gender, increasing age, maternal relative with breast cancer, abnormal genes (BRCA 1, BRCA2 genes), nulliparity, late age at first pregnancy and longer reproductive span (early menarche<12yrs, late menopause>50yrs), obesity, increased dietary fat & alcohol intake, cigarette smoking, previous breast lesion with atypical changes, previous breast cancer.

* Cervical Cancer

It is the 2nd most common cancer in Nigerian women and the most common female genital cancer constituting a major cause of mortality among Nigerian females in their most productive years. It was the commonest cancer reported from Ibadan, Eruwa, Zaria, Jos, Benin and Calabar and in the early years, 2nd to breast in Enugu and Ife-Ijesha4. A steady increase was reported by Babarinsa et al in Ibadan in between 1975-1995 which was attributed to poor screening facillities, and lack of organized national screening programme.

Recent data shows that it has however been overtaken by breast cancer; except in Kano where it was reported as the most common cancer in both sexes (Denny and Quinn, 2006); In Jos, it is the most common female cancer. On the other hand, incidence of other gynae cancers such as choriocarcinoma and endometrial has reduced drastically.

The age range is between 17-80yrs with peak in the 5th decade. Patients are multiparous with average parity of 5.66.5. Multiple marriages, late presentation is common, and majority of the patients have not had Pap smear done before27-28. Squamous cell carcinoma is the most common (9091%) histological type while adenocarcinoma represents 2.4% to 5.1% (de Sanjose, Diaz, *et al.,* 2007).

HPV is a necessary cause of cervical cancer being present in 99.9% of cases30. In a study of 233 cases of cervix cancer from Lagos, HPV 16 and 18 were present in 65.2%31. This supports data that effective vaccination against these 2 types will reduce the cervical burden in Nigeria. It gladdens the heart to know that the Federal Ministry of Health has already given license to bring in vaccines.

Institution of organized screening programmes to detect the pre-cancerous stage has reduced the mortality and morbidity of this cancer in developed countries. This can also be done in Nigeria with strong commitment. A cheaper method by using VIA has been reported to be acceptable and effective.

* Prostate Cancer

It is the most common cancer in Nigerian males; having overtaken liver cancer. It accounts for 6.1-19.5% of all cancers and incidence is increasing. Current data from most parts of the country show it to be the 3rd most common cancer except in Calabar where a very high figure was recorded for prostate cancer as the most common in both sexes accounting for 34.7% of all cancers. Earlier report from that center between 1979-1988 had recorded 28.6% of all male cancers. The increase incidence has been attributed to introduction of PSA screening test which enable earlier diagnosis of case.

Compared to African American men, Nigerian men are 10 times more likely to have prostate cancer and 3.5 times more likely to die from it. Environmental and most importantly, genetic factors have been incriminated as the reason for the geographic differences in incidence.

Risk factors for prostate cancer-

race, age (above 40years), positive family history, high fat diet and high serum androgens levels; the latter being most consistent.

Diagnosis & Treatment-Prostate CA

Digital rectal examination and biopsy, transurethral ultrasound and serum PSA assay, Treatment is by orchidectomy (with the use of anti-androgens); Response is poor as initial response is short lived.

* Colo-rectal Cancer

Colorectal carcinoma is the commonest malignancy of the gastrointestinal tract worldwide. Previous studies had shown it to be a rare disease in Nigeria representing 3-6% of all malignant tumours in most studies. It accounts for 10-50% of all GIT malignancies in Nigeria. Peak incidence-60-70yrs; mean age in Lagos is 50-70yrs. When it occurs in the young, associated with polyposis syndrome or ulcerative colitis should be suspected.

Contrary to previous report which showed it to be rare, recent report shows the incidence to be increasing; an 81% increase over a period of two decades was reported from Ibadan. A recent study from Lagos & Sagamu showed similar trend with an increase in annual frequency of this cancer from 14 cases/annum to 32.3cases /annum39. The low incidence in Nigerians was attributed to fiber rich diet which is common practice and rarity of the familial polyposis syndrome and IBD. Recent urbanization/civilization has resulted in upsurge of confectionary food outlets in major cities resulting in many Nigerians changing their dietary habit from a fiber rich diet, which was common practice to a highly refined carbohydrate and fat diet.

The age incidence of CRC in Nigeria is lower compared to developed countries; about 10 years difference has been reported in many studies. Peak age reported from Nigeria ranged between 42.9yrs to 53yrs with a mean of 46yrs. There has also been an increase in the proportion of young patients with CRC. Reports from various parts of Nigeria showed that 35-42% of CRC are below age 40yrs. CRC in younger age has been shown to present a diagnostic and therapeutic problem and prognosis tend to be less favorable. Generally, CRC is more common in males than females with average male: female ratio of 1.5:1 in Nigeria and 2:1 in America.

Like reports from other parts of Nigeria, recent report from Lagos showed a mean age of 50.7yrs, M: F ratio of 1.3:1 with 23% occurring below 40yrs. The majority (76.4%) was well to moderately differentiated adenocarcinoma. Mucinous carcinoma (10.7%) and signet ring carcinoma (1.2%) were more common in patients under 40yrs compared to well differentiated tumors. Majority of CRC are in the rectosigmoid. Majority of patients present with late stage disease. Management has not improved beyond surgery with or without adjuvant chemotherapy.

Major predisposing factors of CRC

Pre-malignant conditions such as- Polyps, inflammatory bowel disease (IBD) and Dietary factors: Low content of un-absorbable vegetable fiber, High content of refined CHO in diet, High content of animal protein, High fat content in diet, low intake protective micro nutrients, Familial adenomatous polyposis coli syndrome-multiple adenomatous polyps throughout the GIT due to mutation in APC gene on chromosome 5q21. HNPCC (hereditary non-polyposis coli cancer syndrome) or Lynch syndrome Autosomal dominant disorder described by Henry Lynch. Characterized by increased risk of colon cancer and endometrial & ovarian cancer. Caused by mutation in DNA mismatch repair genes, microsatellite instability.

* Liver cancer

Liver cancer is the most common cause of cancer death in Nigeria and most common liver malignancy in Nigeria is hepatocellular carcinoma (HCC). Data from various parts of Nigeria show that it accounts for between 1.6%-7.2% of all cancers in both sexes and represent the 2ndor 3rdmost common cancer in males. HCC was earlier reported to be the most common male cancer until recently when was overtaken by prostate cancer. It is the most common malignancy on medical wards44, 43. It is the most common cause of liver disease in Nigeria accounting for between 29.3% -64% of all liver biopsies in several studies.

The peak age incidence is between the 4th and 5th decade with M: F ratio of 2 to 1. The peak age incidence has been found to be a decade earlier than for liver cirrhosis and hepatitis. A significant number of cases occur in association with liver cirrhosis

Major aetiological factors of HCC

chronic hepatitis B & C virus infections, male gender, exposure to aflatoxin and chronic alcohol abuse; the most prevalent in Nigeria being hepatitis B virus and aflatoxin. Prevalence of HBsAg in serum of Nigerian HCC patients varies between 50-61% 44,50. HCV infection is less with anti-HCV prevalence in serum of HCC patients being 12% -18.7%.

* Childhood Cancer

About 50% of patients seeking medical attention in many general hospitals in Nigeria are children and majority of them suffer from preventable diseases. Previous autopsy study from Lagos revealed that 39.7% of childhood deaths are due to infective causes, only about 3.3% of deaths were attributed to neoplasm. However, with improved child survival due to improved immunization against childhood infections and improved management modalities, the role of malignancies in childhood mortality is becoming more apparent.

Data from various parts of the country show that the five most common childhood cancer are Non-Hodgkin’s lymphoma majority of which are Burkitt’s lymphoma, Retinoblastoma Nephroblastoma, Sarcomas and Leukaemia. Earlier studies from Ibadan had also reported remarkable percentage of brain tumours and leukaemias.

Burkitt’s lymphoma (BL) which is strongly associated with malaria, Epstein Barr virus and malnutrition has higher frequency in the southern forest areas compared to the northern savannah areas. The recent decrease noted in the incidence of BL has been attributed to improved living condition and better malaria control. While retinoblastoma and nephroblastoma are common under 5years, lymphomas and sarcomas occur in older children.

The challenges of childhood cancers-

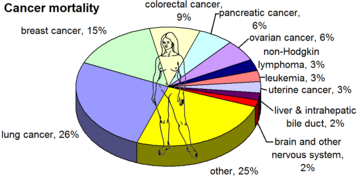
Probability of second malignancy after irradiation e.g. leukaemias and thyroid cancers; Unavailability of immunocytochemistry and other modern diagnostic modalities pose diagnostic challenges as many of these tumour histologically appear as small round blue undifferentiated cells on light microscopy; Poor management outcome due to late presentation, poverty and unavailability of radiotherapy. Although >70% of childhood cancer is now curable with best modern therapy, the treatment is expensive and majority of children (80% of world’s children) currently have little or no access to it in economically disadvantaged countries like ours.

***Cancer in women***

Deaths in Nigerian women were from obstetric complications and communicable diseases; cancer was less common thus the emphasis was on communicable diseases. Data from Ibadan showed common female cancers in 1960-1969 as cervix, breast, NHL; In 1998, breast became the commonest followed by cervix and ovary4-6. Current data shows that female cancers account for about half of the total. The common female cancers reported from the North are cervix, breast, ovary while from Enugu and Lagos breast is commonest followed by cervix both accounting for over 40%.

Five most common female cancers are- breast, cervix, ovary, colorectal and uterus.

***Cancer mortality***

****** Fig 3. Cancer mortality rate in Africa.

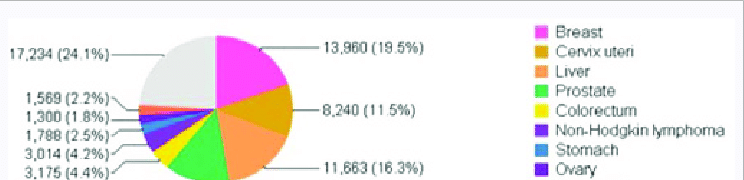
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Fig 4. cancer mortality rate in Nigeria.

***Conclusion***

Although available data are hospital based, it is obvious that cancer incidence is rising in Nigeria. However, majority of the Common Cancers are preventable or curable if detected early. It is noted that the NCCP has set laudable goals; my recommendation is that its activities should be stratified and prioritized; starting from increase public awareness and Training of personnel and provision of up-to-date facilities for cancer registration which will improve data collection by Cancer Registries. When the actual burden of cancer is known, the government will be able to plan more accurately.

It should be noted also that increase awareness will result in increased number of new cases, thus training and re-training of health personnel concerned with Cancer diagnosis and management cannot be over-emphasized. A National Cancer Institute is mandatory which will promote Research and Training in Cancer. Basic treatment of cancer should be part of NHIS. The various challenges that contribute to cancer morbidity and mortality in Nigeria cannot be tackled by govt alone, it should be regarded as everybody’s problem as ‘it represents a tremendous burden on patients, families and the society’.

**INVOLVEMENT OF ANGIOGENIC GENES IN THE DEVELOPMENT AND PROGRESSION OF OSTEOSARCOMA.**

***Introduction***

Osteosarcoma is a malignant tumor of mesenchymal origin and primarily occurs in children, adolescents, and young adults. This pleiomorphic tumor of the bone, based on animal model systems (Gorlick, Anderson *et al.,* 2003), depends on new blood vessel development, also known as angiogenesis, for tumor growth and metastasis. Although modern multimodality treatment has significantly improved tumor resectability and the long-term outcome of these patients, 25–35% of patients with initially non-metastatic disease subsequently develop metastasis and this remains the major cause of death (van Maldegem, Bhosale, *et al.,* 2010). Osteosarcoma is the most prevalent malignant bone tumor ([Meyers and Gorlick, 1997](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B69)), accounting for 30–80% of primary skeletal sarcomas ([Link and Eilber, 1989](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B59)). The peak incidence is at age 10–30 years ([Bielack and Bernstein, 2005](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B10)). Osteosarcomas predominantly target the long cylindrical bones, including the knee joint (approximately half of observations) and the humerus ([Mankin et al., 1996](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B66)). Among the most affected are the femur, tibia, humerus and, less frequently, shoulder blade, and bones of the pelvis and skull ([Enneking et al., 1980](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B25)). Approximately 20% of patients present lung metastases at initial diagnosis and, additionally, in 40% of patient’s metastases occur at a later stage. Eighty percent of all metastases arise in the lungs, most commonly in the periphery of the lungs, and exhibit resistance to conventional chemotherapy ([Kager](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B52) *[et al.,](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B52)* [2003](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B52); [Harting and Blakely, 2006](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B41); [Bacci *et al.,* 2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B6); [Bielack et al., 2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B11); [Hughes, 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B47); [Messerschmitt et al., 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B68); [Posthuma DeBoer *et al.,* 2011](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B78)). The 5-year survival rate for OS patients with metastases is 20%, compared to 65% for patients with localized disease, and most deaths associated with OS are the result of metastatic disease ([Bielack](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B13) *[et al.,](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B13)* [2002](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B13); [Eccles and Welch, 2007](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B24); [Rodriguez *et al.,* 2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B82); [Pradelli *et al.,* 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B80)). Survival of patients has improved with the discovery of new chemotherapies ([Chou and Gorlick, 2006](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B17); [Longhi et al., 2006](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B61); [Perbal *et al.,* 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B76); [Savitskaya *et al.,* 2012](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B88)). The new biotechnological and pharmacological research is directed on the use of markers serum tumor for treatment of osteosarcoma. Several international study groups started a multicenter study on the tumor markers ([Fuchs *et al.,* 1998](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B31); [Ham et al., 2000](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B39); [Woodgate, 2000](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B107); [Lewis and Nooij, 2003](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B56); [Bielack *et al.,* 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B12); [Franke *et al.,* 2011](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B30)).

***Genetic Markers***

[Perbal *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B77) evaluated the expression of CCN1 genes, CCN2, and CCN3 in osteosarcoma; the author finds a synchronous and ordered expression of these genes during osteoblast differentiation in patients with osteosarcoma. In addition, the author affirms that CCN1 and CCN2 genes haven’t a role in the prognosis. In contrast, assessment for CCN3 expression levels at diagnosis may represent a useful molecular tool for early identification of patients with different prognoses ([Perbal](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B77) *[et al.,](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B77)* [2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B77)).

[Gillette *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B34) investigated a human osteosarcoma cell line (OS 99-1), demonstrating the potentiality of this cell line to form *in vitro* calcium nodules upon exposure to mineralization inducing conditions and to represent an osteoblastic rather than chondroblastic OS by displaying several osteoblast-specific markers including collagen I, BSP, OC and ALP, whereas chondroblastic markers (aggrecan and LINK) were undetectable. However, this cell line provides a useful tool for investigating the molecular mechanisms contributing to osteosarcoma and may have the potential to serve as a culture system for studies involving bone physiology ([Gillette *et al.,* 2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B34)).

[De Nigris *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B20) found that Y1 and VEGF/CXCR4 seem to intervene in the pathogenesis of the malignant phenotype of osteosarcoma by acting on cell invasiveness and metastasis growth, because the deletion of the gene produces a lower involvement of the cells by the tumor and a lower spread.

[Zuffa *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B121) investigated the effect of TP53 (tumor protein 53, p53) on the genetic stability in patients with osteosarcoma. The author notes that the protein protects the DNA of cells, although are not known the mechanisms of protection exactly ([Zuffa](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B121) *[et al.](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B121)*[, 2008](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B121)).

[Wei *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B105) analyzed the c-kit gene in patients affected by osteosarcoma; the author notes that gene alteration of the protein is a prognostic marker of the tumor; moreover, exons 11 and 17 can’t be considered for the treatment of cancer through reduction of c-kit tyrosine kinase activity.

[Nathan *et al.,* (2009)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B72) observed, through a molecular analysis of cell line OS1, a genome instability after mutations that affect the function of the transcription factor Runx2. The author asserts that this cell line allows to identify possible molecular abnormalities that transform the osteoblast into cancer cells ([Nathan *et al.,* 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B72)).

The role of transcriptional regulators Oct-4 in osteosarcoma has been studied by [Levings et al. (2009)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B55). According to the author this genetic marker plays a biological role in the proliferation and spread of cancer ([Levings et al., 2009](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B55)).

The expression of Mir-34s was reviewed by [He *et al.,* (2009)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B42) into two categories of osteosarcoma: U2OS (p53 +/+) and SAOS-2 (p53 -/-). The author states that the action of miR-34s is p53-dependent, and causes alteration of proliferation and apoptotic process. [He *et al.,* (2009)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B42) observed a reduced expression and inactivation of miR-34 gene in a group of osteosarcomas.

[Yang  *et al.,* (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B111) evaluated the role of the WWOX gene and, through comparative genomic hybridization, showed how the elimination or downregulation affects the survival of patients with osteosarcoma; also the author believes that the phenotypic alteration affects the early phases of the disease.

Analyzing blood samples of 168 osteosarcoma patients, [Hu *et al.,* (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B45) used PCR amplification and DNA sequencing to determine the TGFBR1∗6A variant, a dominant polymorphism of the transforming growth factor β receptor 1 (TGFBR1). Their conclusion was that TGFBR1∗6A is associated with increased susceptibility to, and metastasis diffusion of, osteosarcoma ([Hu *et al.,* 2010](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B45)).

[Mirabello *et al.,* (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B70) report an association study of common single-nucleotide polymorphisms (SNPs) across 8q24 to explore the role this region may play in osteosarcoma risk. The 8q24 chromosomal region contains several loci that are associated with the risk of many different cancers. The study suggested that several SNPs in 8q24 may be associated with osteosarcoma, but the susceptibility observed was modest ([Mirabello](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B70) *[et al.,](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B70)* [2010](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B70)).

Bcl-xL, a member of Bcl-2 protein family functioning as dominant regulators of apoptotic cell death, has been reported to play important roles in malignant transformation and tumor development. [Wang *et al.,* (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B103) studied the expression of Bcl-xL in osteosarcoma with therapeutic aims. The author, through many genetic techniques, compare the expression of this gene in tumor and non-tumor cells. An increase of Bcl-xL mRNA was observed in cancer cells with metastases compared to non-metastatic cells; also the expression of gene was significantly higher in tissues with tumor compared to healthy ones. Therefore, the author believes that the increase of the expression of Bcl-xL mRNA has a role in the diffusion of osteosarcoma and can be a useful molecular target for the treatment ([Wang et al., 2010](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B103)).

Based on many papers reporting that the reduction of expression of Fas protein is correlated to a higher risk of lung metastases and arsenic trioxide (ATO) may promote cell apoptosis in cancers. [Yang *et al.,* (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B110) evaluated the role of ATO and the degree of expression of the Fas protein of human osteosarcoma cells (Saos-2 cell line); the author affirms that ATO reduces cell proliferation according to the dose and time and increased the expression of the Fas protein, although other mechanisms are interested in this process ([Yang *et al.,* 2010](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B110)).

[Wang *et al.,* (2011)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B101), assert that the + 49G/A polymorphism of cytotoxic T-lymphocyte antigen-4 (CTLA-4), a molecule that decreases the immune response mediated by T-cells, promotes the development of osteosarcoma.

[Lockwood *et al.,* (2011)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B60) performed an analysis of DNA in osteosarcoma tumor samples. The overexpression of cyclin E1 was linked to potential prognostic and therapeutic implications ([Lockwood *et al.,* 2011](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B60)).

A study performed by the Massachusetts General Hospital group asserts that miR-199a-3p is involved in proliferative process of osteosarcoma. The restoration of this marker may provide therapeutic benefits in osteosarcoma. MicroRNAs (miRNA, miR) play an important role in cancer cell growth and migration. However, the potential roles of miRNAs in osteosarcoma remain largely uncharacterized. By applying a miRNA microarray platform and unsupervised hierarchical clustering analysis, they found that several miRNAs have altered expression levels in osteosarcoma cell lines and tumor tissues when compared with normal human osteoblasts. Three miRNAs, miR-199a-3p, miR-127-3p and miR-376c, were significantly decreased in osteosarcoma cell lines compared to osteoblasts, whereas miR-151-3p and miR-191 were increased in osteosarcoma cell lines ([Duan](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B23) *[et al.,](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B23)* [2011](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full" \l "B23)) (Figure 1).

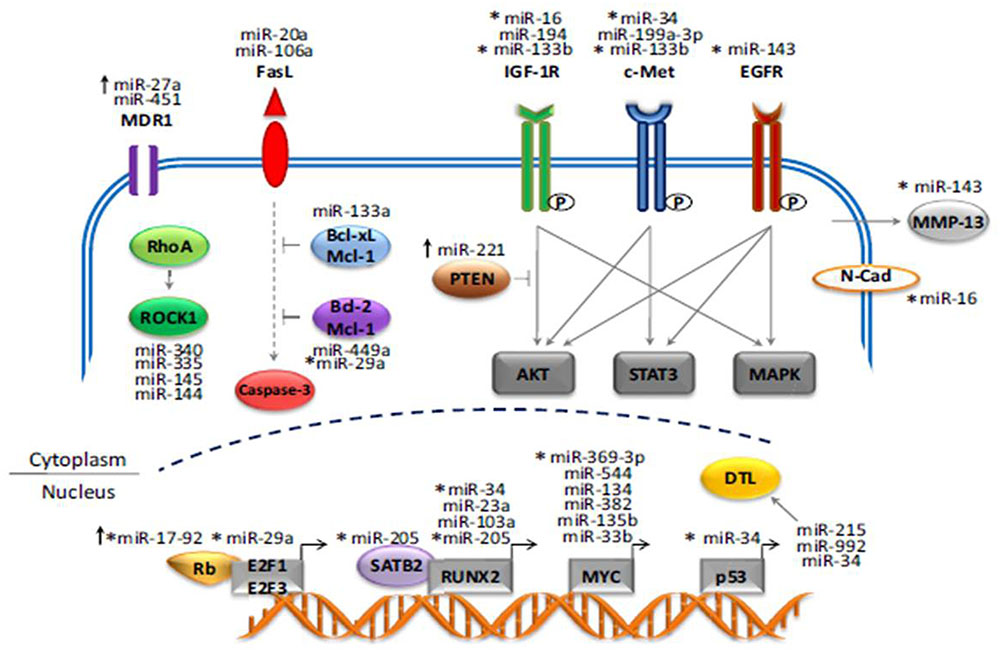


Fig 1*. MiRNA genes that play a role in the development and progression of Osteosarcoma.*

A preliminary study by [Folio *et al.,* (2011)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B29) supports the hypothesis that overexpression of Cortactin (CTTN) gene, contained in the 11q13 amplicon, is involved in osteosarcoma carcinogenesis. The potential of cortactin overexpression as a biomarker for osteosarcoma is, in fact, consolidated and transcriptomic profiling has shown cortactin to be overexpressed in pediatric osteosarcoma. The CTTN represents, according to the author, a valid biomarker for cancer.

[Shen *et al.,* (2012)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B92) examined the expression and localization of hArgBP2 in osteosarcoma MG-63 cells. After the successful construction of a recombinant plasmid of hArgBP2 gene, they identified the expression of GFP-hArgBP2 fusion mainly localized in the cytoplasm and perinucleus of MG-63 cells. The protein was identified and isolated using GFP antibody.

[Yang *et al.,* (2011)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B112), using microarray-based comparative genomic hybridization (aCGH), reported for the first time That vascular endothelial growth factor (VEGF) pathway genes, including the VEGFA protein, is overexpressed and play a role in poor prognosis and reduced survival in osteosarcoma.

[Chen *et al.,* (2012)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B16) used immunohistochemical staining in serial sections of the osteosarcoma analyzed to investigate expression patterns of IGF2 and IMP3 and their relationship with angiogenesis in the tumor. The author states that the increased expression of IGF2 and IMP3 can promote tumor angiogenetic processes.

***Other Genetic Markers***

[Douglas *et al.,* (2008)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B22) investigated whether tumors of the Ewing sarcoma family express the BMI-1 gene, and whether it functions as an oncogene in this highly aggressive group of bone and soft tissue tumors. Their data showed that BMI-1 is highly expressed by ESFT cells and that, although it does not significantly affect proliferation or survival, BMI-1 actively promotes anchorage independent growth *in vitro* and tumorigenicity *in vivo*. The authors, moreover, found that BMI-1 promotes the tumorigenicity of both p16 wild-type and p16-null cell lines, demonstrating that the mechanism of BMI-1 oncogenic function in ESFT is, at least in part, independent of CDKN2A repression. The data suggests a pivotal role for BMI-1 in ESFT pathogenesis.

A recent statistical methodology, called ICAN, allows to select genes that play a role in the prognosis and progression of tumors in studies with small samples, was reported by [Bennani-Baiti et al. (2010)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B9) and tested in Ewing’s sarcoma (ES). Through this methodology the author affirms that CXCR4 expression increases the risk of tumor metastases, while CXCR7 expression is associated with shorter survival.

Some studies have shown that the EWS-ETS fusions, due to genetic changes, does not have a prognostic significance or Ewing’s sarcoma. Consequently, in an integrative genomic study of 105 ES tumors, [Mackintosh *et al.,* (2012)](https://www.frontiersin.org/articles/10.3389/fphar.2017.00150/full#B65) affirms the prognostic significance of 1QG and CDT2 expression in cancer, noting that this marker can be used in tumor therapy selectively.

## ***Conclusion***

During growth the most prevalent kind of tumor are osteosarcoma, chondrosarcoma, and sarcoma of Ewing. Current knowledge of the molecular biomarker involved in each type of tumor has led to better approaches in the treatment. Molecular markers increase the accuracy of the diagnosis and assist in subtyping bone tumors, thus improving the quality of life of patients. This review had the objective to summarize the biomarkers are more interested in these three types of bone tumors.

## ***Future Perspectives***

Despite the large progress in the field, as the discovery of new markers (sCD30 and sCD40L), many challenges are still ahead. The search for biomarkers for non-invasive diagnosis of bone tumors is currently an area research interest. One of the most important aspects will be the identification of microRNAs involved in bone tumors. MicroRNAs are a group of small non-coding RNAs circulating in blood of patients, that play a role in post-transcriptional gene expression in normal and disease physiologies, even if its molecular mechanisms still remain elusive. MicroRNAs have been considered potential biomarkers and can be used for diagnosis, prognosis, and targeted treatment of neoplastic bone disease.

The differential expression profiles of miRNAs can be used as promising diagnostic and prognostic biomarkers of osteosarcoma, chondrosarcoma, and Ewing sarcoma; miRNAs play a positive role in the progression of bone tumors by regulating proliferation, invasion, metastasis, apoptosis and angiogenesis. The identification of more specific non-invasive biomarkers could help the treatment of bone tumors in the future.

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