Demonstration of Amyloid in Osteosarcoma

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Abstract: This study was carried out using 36 paraffin block specimen collected from bone biopsies diagnosed in National Orthopaedic Hospital Enugu. Twenty four (24) of this blocks representing 66.6 % were from male patients while the other twelve (12) representing 33.3 % were from female patients. All the cases were diagnosed as osteosarcoma between January 2010 and June 2016. Five (5) out of the 36 blocks were used as controls. Duplicate (2) sections were made from each block and stained with H &E as well as Highman’s congo red technique. In demonstrating amyloid deposit, in the 36 test samples, 13.8% were positive for amyloid with H &E while 11.1% of the total 36 test samples were positive for amyloid with Highman’s Congo red technique. This test result shows a low incidence of amyloidosis in osteosarcoma. In addition, Congo red showed greater specificity, eliminating false positive result, hence a better stain in all amyloid study.

Keywords: Amyloid, Osteosarcoma, Congo red.

INTRODUCTION

Osteosarcoma is the most well-known harmful bone tumor [1, 2]. It is an old infection that is still not completely comprehended. Osteosarcoma is thought to emerge from crude mesenchymal bone-framing cells, and its histologic trademark is the creation of dangerous osteoid. Other cell population may likewise be available, as these sort of cells may likewise emerge from pluripotent mesenchymal cells, yet any region of dangerous bone in the sore sets up the conclusion as osteosarcoma.

Osteosarcoma is a deadly kind of musculoskeletal harmful development that most typically makes patients die as a result of aspiratory metastatic contamination. [3-7] Most osteosarcomas arise as solitary bruises inside the fastest creating regions of the long bones of adolescents. The primary three impacted part are the distal femur, the proximal tibia, and the proximal humerus, yet basically any bone can be affected.

Chest x ray of patient with osteosarcoma whose death occur because of respiratory metastatic sickness. You can take note of the presence of a pneumothorax and bone-shaping metastatic sores.

Not all osteosarcomas have same clinical introductions. Different objections may get evident inside a period of around a half year (concurrent osteosarcoma), or various districts may be noted over a period longer than a half year (metachronous osteosarcoma). [5] Such multifocal osteosarcoma is emphatically remarkable, anyway when it occurs, it occurs in youngsters more youthful than ten years.

The foundation of treatment is cautious removal of the hazardous injury. Routinely, limb saving (extremity saving) strategies can be used to treat patients with this ailment and, thusly, shield work. Chemotherapy is similarly
needed to treat micrometastatic disease, which is accessible yet often not perceptible in numerous patients (~80%) at the hour of finding [8].

Osteosarcoma is a bone tumor and can occur in any bone, generally in the utermost purposes of long bones close metaphyseal improvement plates. The most notable regions are according to the accompanying: Femur (42%, 75% of which are in the distal femur), Tibia (19%, 80% of which are in the proximal tibia), Humerus (10%, 90% of which are in the proximal humerus), Skull and jaw (8%), Pelvis (8%).

The specific reason for osteosarcoma is obscure. Notwithstanding, various danger factors have been recognized [3-16].

Fast bone development seems to incline people to osteosarcoma, as recommended by the expanded rate during the young adult development spray, the high frequency among huge variety canines (eg, Great Dane, St Bernard, German shepherd), and osteosarcoma's run of the mill area in the metaphyseal territory nearby the development plate (empyphysis) of long bones [3-16].

Hereditary inclination assumes a job. Bone dysplasias, including Paget infection, stringy dysplasia, enchondromatosis, and innate numerous exostoses and retinoblastoma (germline structure) are hazard factors. The mix of sacred transformation of the RB quality (germline retinoblastoma) and radiation treatment is connected with an especially high danger of creating osteosarcoma, Li-Fraumeni disorder (germline p53 change), and Rothmund-Thomson condition (autosomal passive relationship of intrinsic bone imperfections, hair and skin dysplasias, hypogonadism, and waterfalls) [3-16].

The rate is marginally higher in blacks than in whites. Information from the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Pediatric Monograph 1973-2004 are as per the following [17]:

Virchow confirmed in his work that the tincture property of amyloid resemble starch more than cellulose. However, in 1857, Fredrick and Kekule pointed out the high nitrogen content of the livers and spleen diagnosed as Amyloid and this led to the conclusion that amyloid could be albuminous or protein in nature. Amyloid is insoluble and resistant to digestion by proteolytic enzymes but it can be distinguished from other hyaline deposits by its characteristic distribution in some tissue. Also, a variety of histochemical techniques are employed in its demonstration. Amyloid is period acid Schiff (PAS) positive; it stains green or blue with trichrome stain and khaki color with von gieson stain. With iodine or white specimen or histological sections, amyloid is coloured brown and on addition of sulphuric acid may turn violet. Methyl violet; a metachromatic stain turns amyloid rose pink while other tissues are violet. Amyloid is a substance that is characteristically homogenously pink in tissue sections stained with H & E [22]. However, when stained with Congo red, a red or deep pink color is obtained with a light microscope. Also, an apple green birefringence is seen after staining with Congo red in the presence of polarized light.

This research item is aimed at demonstrating the presence of amyloid in Oesteosarcoma.

**MATERIALS AND METHODS**

**Sample Collection**
A total of 36 paraffin tissue block were randomly collected from the National Orthopaedic Hospital Enugu, the hospital was chosen due to their specialties on bones surgeries and treatment. Thirty one (31) tissue blocks were taken from a known diagnosed osteosarcoma between January 2010 and July 2016. The remaining five tissue paraffin embedded blocks served as control which appear normal, certified by qualified pathologist. The paraffin blocks were sectioned at a five (5) microns thickness using a Rotary microtome, the tissues were then stained with Hematoxylin and Eosin (H & E) which was followed by Highman’s Congo red technique, which is known to demonstrate amyloid better than H& E.

Duplicate sections of 5microns was taken made from each block and transferred to a clean grease free slide for staining.

**Highman’s Congo red method**
Congo red form non-polar hydrogen bonds with Amyloid. It is also a fluorochrome and will impart a red fluorescence to amyloids. An important feature of Congo red staining is the red to green birefringence seen when using polarized light. This is due to the parallel alignment of the dye molecules in the linearly arranged amyloid fibrils.
Technique

Stepwise procedure is as followed: Dewax section in xylene, after which the section is taken to water by passing it through descending grades of alcohol (Absolute, 90% and 70%), stain in 0.5% Congo red in 50 percent alcohol for 5 minutes, differentiate in 0.2 percent potassium hydroxide in 80% alcohol for 3 minutes, wash in water, counter stain nuclei in Harris Hematoxylin, wash in water, dehydrate, clear in xylene and mount.

Statistical Analysis

The data obtained in the study were analyzed using SPSS version 20. The results were expressed in percentage (%) frequency.

Results and Discussion

Hematoxylin and Eosin (H & E) Technique

Hematoxylin and Eosin procedure is done according to Omorodion et al. 2018.

All the thirty-one (31) paraffinized tissues (bones) sections were stained with H & E and Highman’s Congo red technique separately.

Table-1: Presence of Amyloid in Osteosarcoma in relation to Age

| Age Range | No of Selection | Positive section |
|-----------|----------------|------------------|
| 01-10     | 2              | 0                |
| 11-20     | 17             | 2                |
| 21-30     | 10             | 2                |
| 31-40     | 1              | 0                |
| 41-50     | 1              | 0                |

| S/N | DATE | LAB NO | AGE | SEX | DIAGNOSIS |
|-----|------|--------|-----|-----|-----------|
| 1   | 2010 | H17    | 21  | M   |           |
| 2   | 2010 | H143   | 19  | M   |           |
| 3   | 2010 | H154   | 14  | M   |           |
| 4   | 2010 | H203   | 21  | M   |           |
| 5   | 2011 | H20    | 18  | M   |           |
| 6   | 2011 | H50    | 31  | M   |           |
| 7   | 2011 | H107   | 27  | M   |           |
| 8   | 2011 | H125   | 18  | F   |           |
| 9   | 2011 | H195   | 23  | F   |           |
| 10  | 2012 | H11    | 21  | M   |           |
| 11  | 2012 | H49    | 17  | M   |           |
| 12  | 2013 | H34    | 8   | M   |           |
| 13  | 2013 | H39    | 18  | M   |           |
| 14  | 2013 | H42    | 15  | F   |           |
| 15  | 2013 | H77    | 10  | F   |           |
| 16  | 2013 | H150   | 42  | F   |           |
| 17  | 2013 | H173   | 17  | M   |           |
| 18  | 2013 | H183   | 19  | F   |           |
| 19  | 2013 | H27    | 20  | F   |           |
| 20  | 2013 | H21    | 14  | M   |           |
| 21  | 2014 | H39    | 19  | M   |           |
| 22  | 2014 | H132   | 11  | M   |           |
| 23  | 2014 | H120   | 29  | M   |           |
| 24  | 2015 | H177   | 13  | F   |           |
| 25  | 2015 | H176   | 12  | F   |           |
| 26  | 2015 | H170   | 18  | F   |           |
| 27  | 2015 | H182   | 21  | M   |           |
| 28  | 2015 | H206   | 14  | F   |           |
| 29  | 2015 | H212   | 23  | M   |           |
| 30  | 2015 | H339   | 16  | M   |           |
| 31  | 2015 | H17    | 21  | F   |           |
Fig-1: Pink arrows showing the presence of amyloid deposit

The result obtained showed that the occurrence of amyloid in osteosarcoma is relatively low in both sexes. However, even with this low distribution, it was found that males were more predisposed to amyloidosis in osteosarcoma than females. These findings may as well be true due to the fact that males are more predisposed to osteosarcoma than females perhaps due to longer period of skeletal growth in comparison to females [19]. The highly predisposed age grade to osteosarcoma is between the ranges of 11-20 followed by 21-30 age grades. These age grade have more positive cases of amyloidosis in comparison to other age [23]. Finally, it was also seen that the percentage frequency of osteosarcoma in males is 65% while that for females is 63.6%. This again is in agreement with findings of Dorfman [24]. Amyloid affects males than females [25].

CONCLUSION

The incidence of amyloidosis in osteosarcoma in the general population is unknown as no research has been carried out so far to determine this.

RECOMMENDATIONS

More research should be carried out to incriminate the specific cause for the deposition of amyloid in osteosarcoma, to ascertain why females are not as predisposed to osteosarcoma as males, funds should be made available to scientist carrying out further research on this topic and lastly all treatment should be given early to prevent degeneration to chronic state.

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**CITATION:** Iyare Goffrey Innocent *et al* (2021). Demonstration of Amyloid in Osteosarcoma. *South Asian Res J Nurs Health Care, 3*(1): 7-11.