ISSN 2664-8059 (Print) & ISSN 2706-767X (Online)
South Asian Research Journal of Nursing and Healthcare
Abbreviated Key Title: South Asian Res J Nurs Health Care

| Volume-3 | Issue-1 | Jan-Feb -2021 | **DOI: 10.36346/sarjnhc.2021.v03i01.003**

Original Research Article

Demonstration of Amyloid in Osteosarcoma

Iyare Goffrey Innocent[1], Omorodion Nosa Terry[2*], Nwibana Barisuka Kiofi[3]

[1]Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, Igbinedion University, Edo state, Nigeria
[2]Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Nigeria
[3]River state College of Health Sciences and Management Technology, Portharcourt, River State, Nigeria

***Corresponding Author**
Omorodion Nosa Terry

Article History
Received: 08.12.2020
Accepted: 11.01.2021
Published: 23.01.2021

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'scongo 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 oocurs 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 uttermost 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 metachrornatic stain turns amyloid rose pink while other tissues are violet. Arnyloid 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.

REFERENCES

1. Marulanda, G. A., Henderson, E. R., Johnson, D. A., Letson, G. D., & Cheong, D. (2008). Orthopedic surgery options for the treatment of primary osteosarcoma. *Cancer Control*, *15*(1), 13-20.
2. Vander Griend, R. A. (1996). Osteosarcoma and its variants. *The Orthopedic clinics of North America*, *27*(3), 575.
3. Peltier, L. F. (1993). *Orthopedics: a history and iconography* (No. 3). Norman publishing.
4. Campanacci, M. (1999). Preface. *Bone and Soft Tissue Tumors: Clinical Features, Imaging, Pathology and Treatment*. 2nd ed. New York: Springer-Verlag.
5. Weis, L.D. (1998). Common malignant bone tumors: osteosarcoma. Simon MA, Springfield D, eds. *Surgery for Bone and Soft-Tissue Tumors*. Philadelphia: Lippincott-Raven. 265-74.
6. Gorlick, R., Janeway, K., Marina, N. (2016). Osteosarcoma. Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*. 7th ed. Philadelphia: Wolters Kluwer. 876-97.
7. MacDonald, M. G., & Seshia, M. M. (2015). *Avery's neonatology: pathophysiology and management of the newborn*. Lippincott williams & wilkins.
8. Kim, S. Y., & Helman, L. J. (2009). Strategies to explore new approaches in the investigation and treatment of osteosarcoma. In *Pediatric and Adolescent Osteosarcoma* (pp. 517-528). Springer, Boston, MA.
9. Clark, J. C., Dass, C. R., & Choong, P. F. (2008). A review of clinical and molecular prognostic factors in osteosarcoma. *Journal of cancer research and clinical oncology*, *134*(3), 281-297.
10. Pochanugool, L., Subhadharaphandou, T., Dhanachai, M., Hathirat, P., Sangthawan, D., Pirabul, R., ... & Pornpipatpong, N. (1997). Prognostic factors among 130 patients with osteosarcoma. *Clinical orthopaedics and related research*, (345), 206-214.
11. Tsuchiya, H., Tomita, K., & Ten-year Intergroup. (1992). Prognosis of osteosarcoma treated by limb-salvage surgey: the ten-year intergroup study in Japan. *Japanese journal of clinical oncology*, *22*(5), 347-353.

12. Taylor, W. F., Ivins, J. C., Unni, K. K., Beabout, J. W., Golenzer, H. J., & Black, L. E. (1989). Prognostic variables in osteosarcoma: a multi-institutional study. *JNCI: Journal of the National Cancer Institute*, *81*(1), 21-30.
13. Hudson, M., Jaffe, M. R., Jaffe, N., Ayala, A., Raymond, A. K., Carrasco, H., ... & Robertson, R. (1990). Pediatric osteosarcoma: therapeutic strategies, results, and prognostic factors derived from a 10-year experience. *Journal of Clinical Oncology*, *8*(12), 1988-1997.
14. Meyer, W. H., Schell, M. J., Kumar, A. M., Rao, B. N., Green, A. A., Champion, J., & Pratt, C. B. (1987). Thoracotomy for pulmonary metastatic osteosarcoma. An analysis of prognostic indicators of survival. *Cancer*, *59*(2), 374-379.
15. Yang, J., Yang, D., Cogdell, D., Du, X., Li, H., Pang, Y., ... & Zhang, W. (2010). APEX1 gene amplification and its protein overexpression in osteosarcoma: correlation with recurrence, metastasis, and survival. *Technology in cancer research & treatment*, *9*(2), 161-169.
16. Kubista, B., Klinglmueller, F., Bilban, M., Pfeiffer, M., Lass, R., Giurea, A., ... & Singer, C. F. (2011). Microarray analysis identifies distinct gene expression profiles associated with histological subtype in human osteosarcoma. *International orthopaedics*, *35*(3), 401-411.
17. Mirabello, L., Troisi, R. J., & Savage, S. A. (2009). Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *115*(7), 1531-1543.
18. Omorodion, N. T., Atoigwe-Ogeyemhe, B. E., Achukwu, P. U., & Odigie, E. B. (2019). Histopathological changes associated with exposure of some viscerals and testicular tissues to bisephenol A and di (2-ethylhexyl) phthalate. *Tropical Journal of Pharmaceutical Research*, *18*(6).
19. Ottaviani, G., & Jaffe, N. (2009). The epidemiology of osteosarcoma. In *Pediatric and adolescent osteosarcoma* (pp. 3-13). Springer, Boston, MA.
20. Quock, T. P., Yan, T., Chang, E., Guthrie, S., & Broder, M. S. (2018). Epidemiology of AL amyloidosis: a real-world study using US claims data. *Blood advances*, *2*(10), 1046-1053.
21. Wang, J., Tanila, H., Puoliväli, J., Kadish, I., & van Groen, T. (2003). Gender differences in the amount and deposition of amyloidβ in APPswe and PS1 double transgenic mice. *Neurobiology of disease*, *14*(3), 318-327.
22. Skinner, M., Anderson, J. J., Simms, R., Falk, R., Wang, M., Libbey, C. A., ... & Cohen, A. S. (1996). Treatment of 100 patients with primary amyloidosis: a randomized trial of melphalan, prednisone, and colchicine versus colchicine only. *The American journal of medicine*, *100*(3), 290-298.
23. Huvos, A. G. (1986). Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. *Cancer*, *57*(7), 1442-1449.
24. Dorfman, M. S. (1998). *Introduction to risk management and insurance*. 清华大学出版社有限公司.
25. Guyatt, G., Walter, S., & Norman, G. (1987). Measuring change over time: assessing the usefulness of evaluative instruments. *Journal of chronic diseases*, *40*(2), 171-178.

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