Patterns of Haemosiderin Deposits in Knee Joint Diseases: The Perl’s Prussian Blue Reaction Method

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Qualitative evaluation of haemosiderin deposits in 40 synovium biopsies obtained in two years was studied at National Orthopaedic Hospital Enugu (NOHE). Thirty-two (80%) from knee joints were paraffin processed and stained with Perl’s Prussian blue reaction method. Patients were grouped into seven age groups in relation to six diseases that occurred, the age group (20-29) was found of highest frequency of nine (28.1%). Twenty (62.5%) and twelve (3.75%) females were affected, and age group (20-29) of males with eight (40%), and (60-70) of females with six (50%) were highest frequencies for both sexes. Six disease conditions were found, synovitis and sub-types occurred in twenty-eight (87.5%) patients. Haemosiderin deposits were graded and scored on an arbitrary scale of none seen, 1+ to 3+ based on intensity of Prussian blue developed. Haemosiderin was indicated in eighteen (56.3%) of all the cases and pattern of depositions were scored as followed: fine score 1+, fine and dense 2+, while coarsely clumped scored 3+. Fourteen (43.3%) cases revealed non-haemosiderin deposit.

Keywords: Knee; Joint; Synovium; Haemosiderin; Deposits; Synovitis.

1. Introduction

Many investigators have researched diseased joints for factors responsible for articular bone damage, and probed the inflammatory process and degenerative changes that arise [1-3]. Trauma and the resultant haemorrhage into the joint space produce iron deposits, which histologically is secreted by the synovial membrane cells and cytologically is found in the synovial fluid as in the A cells and inflammatory cells.

Other conditions in which iron deposits are found include rheumatoid arthritis (RA) [2], osteoarthritis (OA) [3], haemochromatosis [4], haemosiderosis [5], cancer [6], synovitis [7], beta thalassaemia [4], and cyst have had unpredictable degree of knee joint reactions. These also, have been associated with bone deformation, anaemia and other systemic disorders, indicating that iron deposits were found in the synovium or the fluid [8,9].

In all these conditions Fe$^{2+}$ or Fe$^{3+}$ deposits were qualitatively and quantitatively analysed [10,11]. Some of these cases present inflammatory and immunological responses, while clinical anaemia produced in a few others were in part due to iron deposits in joints and elsewhere in the body [7,11]. Also the liberation of free oxygen radical by iron catalyses is said to accelerate high hyaluronic acid degradation, induce lysosomal emzyme release, and promote joint injury at steady degree [12,14].

The immune complexes are known to mediate mono – and poly – morphonuclear cells infiltration in other to provoke further damage by excreting large amount of superoxide ion (O$_2^-$) and hydrogen peroxide ion (OH$^-$), even as extra vascular iron deposit increases in the case of rheumatoid arthritis [2,15]. The participation of iron in lipid peroxidation has been considered a possible mechanism in rheumatoid arthritis and brain tissue damage [3]. There are cases of non-inflammatory changes in the articu-
lar cartilage of joints that result in cartilage destruction, that present secondary synovitis [2,16] by secreted proteases and cytokines. These substances as liberated in synovial cavity initiate varying ranges of clinical symptoms which elicit morphologic changes, that often calls for arthroplasty [17].

The increasing revelation of haemosiderin iron in organ lesions or systemic diseases, joint and bone disorders might interrupt iron metabolism. Implicated human leucocyte antigen (HLA) class I gene is A3, B14, and B7 [18]. The study is therefore designed to detect the present and pattern of iron deposits in the various diseased conditions that occur in the knee joint, and their association with age and sex.

2. Materials and Methods

A total of forty paraffin wax processed sample were selected for the study. Thirty-two of these were knee synovium drawn from National Orthopaedic Hospital Enugu (NOHE). The samples were obtained from patients consulted between 1996 and 1997. The remaining samples were not considered from inclusion in this study because they lacked merits. Two thin paraffin sections of 5µm were cut from each block. One section served as spare and standard Perl’s Prussian Blue Reaction Method was used to stain all alongside control samples.

2.1 Perl’s Prussian Blue Reaction Method
The principle is based on the combination of Potassium Ferrocyanide with Ferric Iron (Fe$^{3+}$) to form the insoluble Prussian blue precipitate as follows.

$$4\text{FeCl}_3 + 3\text{K}_4\text{Fe(CN)}_6 = \text{Fe}_4\{\text{Fe(CN)}_6\}_3 + 12\text{KCl}$$

3. Result

Table 1 shows age and sex distributions of 32 patients in the study. Age group 0-9 years was absorbed of knee diseases, and all others were indicated one or more disease conditions. Individual group involvement shows that five patients or 15.6% were in 10-19 age group, nine patients or 28.1% were in 20-29 age group; seven patients or 21.9% in 30-39 age group; two or 6.25% in 40-49 age group; three or 9.38% were in 50-59 age group; and six or 18.8% were in 60-70 years age group. Hence a total of twenty (62.5%) males and twelve (37.5%) females were observed.

Table 2 shows diseases incidence and the distribution across age groups and sexes. Age 20-29 years was associated with two conditions: acute synovitis and villonodular synovitis. In the group, males affected were eight (88.9%), five for villonodular synovitis, three for acute synovitis while one (11.1%) female was affected with acute synovitis. Four disease conditions were found in age group 30-39 years involving five (71.4%) males and two (28.6%) females, while age group 40-49 years had only acute synovitis occurring in two (6.25%) males. In age group 50-59 years were three lesions, acute synovitis, chronic synovitis and synovioma, which affected two (66.7%) females and one (33.3%) male. Three knee diseases were indicated in age group 60-70 years affecting females, such that chronic synovitis occurred in four (67.7%).

Of the lesions found, myxosarcoma and simple cyst were observed to occur once; synovioma twice traiting behind chronic synovitis found in eight patients. Villonodular synovitis and acute synovitis were of frequency ten each.

The occurrence of haemosiderin iron deposit in eighteen (56.3%) of all patients in the survey, and its pattern of distribution in the tissues are shown in Figures I, II, III, and Table III were as; ten (56.6%) males and (44.4) females.
That is heamosiderin deposits was found in:
- The synovial layer and macrophage.
- The superficial connective tissue layer and
- The deep loose connective tissue layer.

Eleven (61.1%) out of eighteen (56.3%) cases positive show fine pattern, scored 1+, and seven of these were incident on acute synovitis and two each distributed among chronic and villonodular synovitis. Also five (27.8%) were dense pattern, scored 3+ observed in all the synovioma, myxosarcoma, acute and chronic synovitis, while two (11.1%) revealed fine and sense pattern scored 2+.

### Table 2: Frequency of Diseases, distribution in the age group and sex

<table>
<thead>
<tr>
<th>Age Group</th>
<th>10–19</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-70</th>
<th>Frequency of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Synovioma</td>
</tr>
<tr>
<td>M</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Myxosarcoma</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Simple Cyst</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Villonodular Synovitis</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Acute Synovitis</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Frequency in the age sex</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>32</td>
</tr>
</tbody>
</table>

### Table 3: Degree of Iron in Various Knee Joint diseases and Frequency of Occurrence

<table>
<thead>
<tr>
<th>Degree of disposition</th>
<th>No Iron</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Period before surgery in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovioma</td>
<td>2</td>
<td>2</td>
<td>14-192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myxosarcoma</td>
<td>1</td>
<td></td>
<td>6-144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple cyst</td>
<td>1</td>
<td></td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villonodular Synovitis</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>2-120</td>
<td></td>
</tr>
<tr>
<td>Acute Synovitis</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>3-36</td>
<td></td>
</tr>
<tr>
<td>Chronic Synovitis</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3-36</td>
<td></td>
</tr>
<tr>
<td>Total Frequency</td>
<td>14</td>
<td>11</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Other lesions involved are chronic and villonodular synovitis. Negative situations were prominent in fourteen (43.7%) and cut across all the lesions that occurred except in synovioma and myxosarcoma. The duration before clinic and surgery covered a period of 3 to 192 months in all the diseases Table 3.

### 3.1 Clinical History
- Three patients reported to have had childhood knee injury at school received brief medical attention. Two were males in age group 10-19 years and one female of age 20-29 at surgery.

Fig 1: Coarse and dense haemosiderin deposition in the synovioma scored 3+.
4. Discussion

Several diseases of the knee joint are associated with the disease of the Synovium, and the presence of inflammatory cells in all the cases is considered a strong factor for persistent resultant diseases. The constant and frequent demonstration of haemosiderin depositions in the synovium in certain lesions of the knee particularly arthritis, have shown iron secretion in synovial membrane cells, and predict that knee joint diseases could be associated with systemic disorders [19-22].

We found haemosiderin deposits in all the six diseases seen in our study save in one. This is in agreement with previous reports [23-25], which has considered other factors order than iron directly or indirectly in Synovial membrane inflammation. Also different degrees of cellular proliferations were observed at synovial cell layer, superficial connective tissue, and in deep loosed connective tissue, and scanty phagocytic macrophages were present. This observation lacks consistent correlation with duration of disease onset, and was not a common picture with the subtypes of synovitis that occurred in the study.

However two cancer cases revealed synovial cell layer Hyperplasia as previously observed [2] in a case of RA. When this finding is compared with other reports [25-27] it must have supported the opinion that cellular proliferation in synovitis in this study could have been enhanced by iron presence and change in synovial fluid pH toward acidity [25-27].

A total population of 32 knee Synovia were investigated in this study, the age and sex considered among six diseased lesions, had males prevalence over females table I & II. This is unlike past reports where female prevalence was observed with inflammation of joints due to emotional stress and gene [2,3]. Since haemarthropathy of the knee joint is primarily due to trauma and secondarily due to varying degrees of disorder [28], same factors are suggestive causes and had concentrated sufferers in age groups (20-29) and 30-39) years. Also in this group no case of RA or OA was established. Concerning the attention age grouping received with synovium diseases

- Six were homeboys whose minor injuries can be remembered, though never had indication for treatment, and were in age group 20-29.
- Two females in age group 30-39 had repeated medical course association with tropics, and were transfused once or twice before clinical presentation in relation to knee joint disease.
- All others either could not give account of onset or related indications. These were lost immediately after surgery.
was because we noticed a high frequency of synovitis and its subtypes among a significant population within the first to third decade of life. Again we observed a pattern of disease incidence, rising from age group (10-19) years and suddenly decreased from highest frequency of occurrence at (20-29) years down to (40-49) years table II.

This suggests any of the following:

- There is deposition of either iron or calcium pyrophosphate dehydrate (CPPD) with acute clinical manifestation of synovitis as in genetic haemochromatosis complicating arthritis [29-31],
- There is differential degradation of hyaluronate produced by synovial membrane among residental cells, envisaged phagocytic cells and inflammatory superoxide as in arthritic synovium [25,32],
- There is defective immunologic response occasioned by intracellular endocytocized hyaluronate by CD44 receptor with increase presence of CD4 + T cells and complementary reduction in CD8 + T Cells at high infiltration of polymorphonuclear leucocytes in an inflamed joint [33].
- There may be presence of genetic products of HLA dominant inherited in a heterogeneous manner for macrophage system iron secretion as indicated in some reports [15,34]. Some teams of investigators in their different studies had large population compared and had results similar to ours but not on knee synovium alone [2,3,4].

Haemosiderin deposit was demonstrated in eighteen (56.3%) of the general population in study. Ten (55.6%) of these positive cases were males and eight (44.4%) were females. The result if considered in contrast with knee Synovium lesions observed elsewhere haemosiderin iron deposition lacks significant correlation with sex. This findings is at variance with past records [2,3] in which knee diseases occurrence were secondary to systemic lesions or RA and OA. When synovitis and its subtypes are considered, the relevance of iron deposits in sex will be lost, because males and females involved are not of equal frequency. Also sex to haemosiderin deposit correlation observed in acute synovitis is also defective based on same reason. However the result supports the fact that in acute synovitis, haemosiderin deposition is passive, and is not related to duration of knee synovial membrane lesions but is related to the degree of inflammatory infiltrates [3,14,35,36]. Experimental models have shown that ferric Citrate was capable of inducing proliferation of synovium cells DNA [24], and another record [31] insisted that iron presence potentiates multiplication of Synvial type A Cells in arthritis tissue observed for hyaluronate degradation.

From these findings, the presence of iron in knee diseases can mediate the clinical face, and may stimulate the secretion or more iron through phagocytic Synovial type A cells or reduce same by granulocytes. These also support our finding. The observation of iron in villonodular Synovitis is not known to us, though heavy iron deposits were reported in pigmented villonodular Synovitis [13,14], and in haemochromatosis complicated Synovitis. Our finding that two cases revealed finely dispersed and one dense case out of the ten manifested in the study is near insignificance.

In addition, the presence of haemosiderin could be due to capillary leakage [3] which may be associated with rare negative inheritance of HLA – A3. A study observed the presence of this gene in 28% of general population not suffering from haemochromatosis and such might be a possibility in this case. Our finding from chronic synovitis is that of finely dispersed haemosiderin deposits, and is in line with other records [2].

Though certain reports [3,36] were able to confirm heavy deposits, which are conditionally related to the degree of inflammatory lymphocytes and macrophage. Such reports insisted that the presence of iron promoted inflammatory response by the stimulation of polymorphonuclear leucocytes system. In synovitis as well as certain other inflammation of the joints, three mechanisms can individually or in association facilitate iron deposition in the synovial membrane. These are significant mechanical microtraumata [2], capillary iron leakage[3] and invading phagocytes, and alteration of the phagocytic potentials [37].

No haemosiderin deposit was observed in 43.7% of the population, and in all the disease con-
ditions indicated in the study. A case of clear-cut correlation between the histologic subtypes of synovitis and the number of iron deposits was recorded in a report. The difference found was considered independent of clinical diagnosis [3], and the histologic entities enumerated in that study denote chronic and acute situations. Histologic subtypes of synovitis should reveal variantly villonodular, acute and chronic entities. These were present in our findings in which there is no classic relationship between the histologic subtypes of synovitis and quality of iron deposited. The discovery suggests, that the existence of other factors either released by injured synovial cells or introduced systematically capable of inducing knee joint inflammatory diseases either alone or in association with iron. Factors implicated here are degraded hyaluronic acid [31], heparan sulphate proteoglycan [15], increased calcium deposits [2], mercury and manganese deposits [38], phosphophatase A2 and endogenous phospholipase A2, and bacterial toxins like phospholipase C associated with inflamed Synovium [39]. These factors particularly, excess deposition of calcium pyrophosphate has been incriminated in particular cartilage joint damage to produce arthritis from acute synovitis [14]. Further studies are needed to prove other secretory entities of synovitis.

5. Conclusion

The study has revealed that considerable iron deposits may or not be present in synovitis and its subtypes even in other knee joint synovium diseases as most depositions were passive. Iron deposits in synovial membrane may often potentiate inflammatory processes singly or in association with other factors. There is no recognizable correlation between synovial membrane haemosiderin deposition and duration of disease onset.

References


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