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A CLINICOPATHOLOGIC STUDY OF CHRONIC SUBDURAL HAEMORRHAGE WITH SPECIAL EMPHASIS ON COX-2 AND VEGF EXPRESSION

Pathology

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ABSTRACT

Introduction: Chronic subdural haemorrhage is known for high recurrence rates. Recently, VEGF and COX2 have been implicated as causal agents for recurrences, which can be targeted to prevent repeated surgeries and reduce morbidity.

Material and Methods: Retrospective study including all cases (n=40) of chronic subdural haemorrhage from 2013 to 2017. The membranes were classified into four histologic subtypes. COX 2 and VEGF antibodies were applied on all cases. A p value of <0.05 was considered statistically significant.

Results: Four subtypes were type 1: non inflammatory (22.5%), type 2: inflammatory (20%), type 3: haemorrhagic inflammatory (27.5%) and type 4: scar inflammatory (30%). Glasgow coma score less than 13 (30%), maximum mean thickness of subdural haemorrhage (6.88), highest VEGF positivity rate (100%) and highest COX 2 positivity rate (91.67%) were found in type 4 membrane [p value 0.003 and 0.0001].

Conclusion: We found that type 4 membranes had worst Glasgow coma score, maximum thickness on CT, highest positivity for VEGF and COX2. Therefore, these are likely to have maximum recurrences and hence can be targeted for anti COX 2 and VEGF therapy.

KEYWORDS

Subdural; Membrane; Hematoma; COX2; VEGF; Inhibitors

INTRODUCTION

Chronic subdural haemorrhage is a neurosurgical condition which develops within four to eight weeks after head trauma.(Tanaka and Ohno, 2013) It is notorious for recurrences even after surgical treatment with a recurrence rate as high as 20-30% .(Stroobandt et al, 1995) Although, many theories have been proposed for explaining its pathophysiology, very little is known about its clinicopathologic features. (Ito et al, 1978) Very few authors have studied histopathology of subdural membrane or tried to correlate histology with clinical features. (Ito et al,1978; Nagahori et al,1993; Park M et al,2015) A possible role of COX 2 and VEGF in the pathogenesis of chronic subdural haemorrhage has been put forward recently and these have also been implicated as the causal factor for the recurrences. (Suzuki et al,1999;Weigel et al,2001; Hohenstein et al,2005; Cianchi et al,2001) This can lead to a novel pharmacotherapy in the form of COX2 and VEGF inhibitors which will reduce recurrence rates and hence morbidity. Therefore, we conducted this study with the aims to elaborate the clinico histopathologic features of chronic subdural haemorrhage from a tertiary care centre in North India, and analyse immunohistochemical expression of COX2 and VEGF in the subdural membranes.

MATERIAL AND METHODS

A retrospective study was carried out at the Department of Pathology, PGIMER & Dr RML Hospital, New Delhi including all cases (n=40) of chronic subdural haemorrhage over a span of five years from 2013 to 2017 . The clinical details of the cases were retrieved from medical records section and the histopathology slides from department of pathology. All the histopathology slides were reviewed and classified into four subtypes: type 1: non inflammatory, type 2: inflammatory, type 3: haemorrhagic inflammatory and type 4: scar inflammatory. (Nagahori et al,1993) Type1 membrane (non inflammatory) is composed of a single layer of immature fibroblasts and collagen with only mild inflammatory infiltrate. Type 2 membrane (inflammatory) is composed of single layer of connective tissue but shows dense inflammatory infiltrate and neoangiogenesis. Type 3 (haemorrhagic inflammatory) membrane is composed of two to three layers of connective tissue with extensive haemorrhage into the membrane. Alongwith this many large calibre vessels are seen on dural side of this membrane and small calibre capillaries on hematoma cavity side. Type 4 (scar inflammatory) membrane is composed of cicatricial tissue which may be showing inflammation and haemorrhage. COX 2 antibody [rabbit monoclonal, SP 21,prediluted, Biogenex] and VEGF antibody [rabbit polyclonal, prediluted AR483-5R, Biogenex] were applied on all cases using polymer method. Antigen retrieval was done in TRIS EDTA buffer (pH=9). Statistics were applied using statistical package for social sciences (SPSS) version 21.0. Quantitative variables were compared using Kruskal Wallis Test and qualitative variables were correlated using Chi Square test. A p value of <0.05 was considered statistically significant. On immunohistochemical analysis intensity was scored as mild, moderate and strong. Proportion of cells staining was categorized as 1+ to 3+ (<10%, 10-50% and > 50%).

RESULTS

Age range varied from 10 years to 85 years. Out of the 40 cases, 30 were males and ten were females (M:F-3:1). The most common site was fronto-temporo-parietal region. The two most common etiologies were trivial trauma followed by use of oral anticoagulants. Clinical features varied from trivial symptoms like headache (n= 17), vomiting (n= 8) to more serious presentations like altered sensorium (n= 18), loss of consciousness (n= 4), weakness of limbs (n= 17) and focal neurological deficits (n= 9). GCS scores (available in 30 cases) varied from 7 to 16. Hematoma thickness of subdural haemorrhage on Computerized Tomography (CT) scans (available in 14 cases) ranged from 0.8 cm to 13cm. The subtype wise distribution of clinical features is as shown in table 1.

TABLE 1 : Demographic features and presenting complaints according to subtypes

Subtype	Age	Sex	GCS	Thickness on CT	Headache	Vomiting
Type 1	10-85y	M-8,F-1	14-16	1.24-4 cm	6	1
Type 2	38-79y	M-6,F-2	9-15	1.4-2.8cm	3	2
Type 3	18-74y	M-7,F-4	7-15	3-11cm	4	2
Type 4	16-79y	M-10,F-2	10-15	0.8-13cm	5	3

The subdivision of the cases into four subtypes was as follows: type1: non inflammatory (22.5%), type 2: inflammatory (20%), type 3: haemorrhagic inflammatory (27.5%) and type 4: scar inflammatory (30%) [Fig 1 to 4].(Nagahori et al,1993)

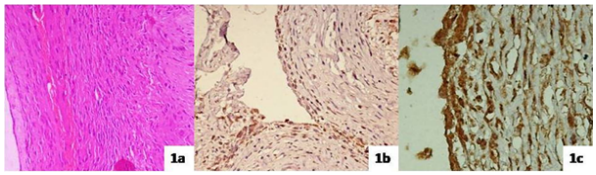


FIGURE 1

Figure 1: Type 1 (Non Inflammatory membrane);1a: [H&E, 200x],1b:COX-2 [IHC, 200x] 1c: VEGF [IHC, 400x]

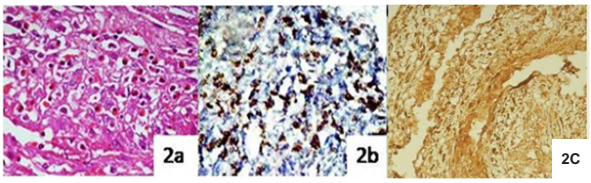


FIGURE 2
Figure 2: Type 2 (Inflammatory membrane) ; 2a: showing many eosinophils [H&E, 400x], 2b: COX-2 [IHC, 400x], 2c: VEGF [IHC, 200x]

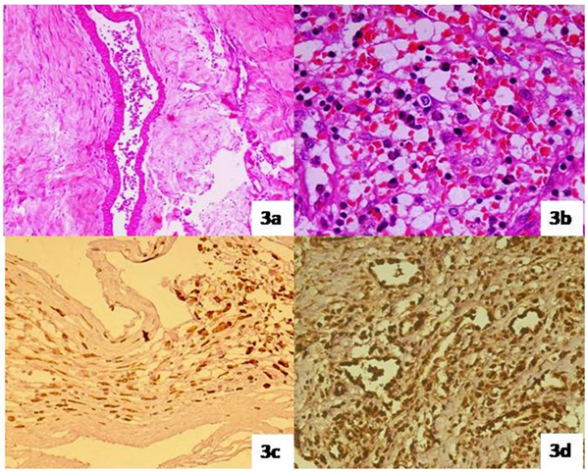


FIGURE 3
Figure 3: Type 3 (Haemorrhagic Inflammatory membrane) ;3a: showing large calibre vessels [H&E,100x], 3b: showing haemorrhage [H&E,400x] 3c: COX-2 [IHC,200x], 3d: VEGF [IHC,200x]

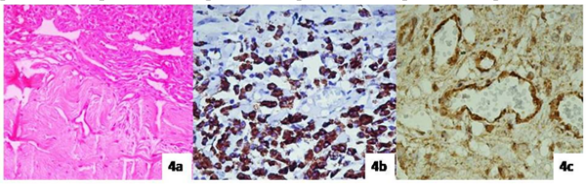


FIGURE 4
Figure 4: Type 4 (Scar Inflammatory membrane); 4a: [H&E, 200x], 4b: COX-2 [IHC, 400x], 4c: VEGF [IHC, 400x]

Detailed histopathological features were studied in all the cases noting the type of infiltrate, presence of eosinophils, the intensity of infiltrate, the presence of hemosiderin and calcification (Table 2 , 3,4).

TABLE 2 : Distribution of intensity of infiltrate

Subtype/Intensity	Mild	Moderate	Dense	Total
Type 2	1	6	1	8
Type 3	3	5	3	11
Type 4	Nil	9	3	12

TABLE 3 : Distribution of type of infiltrate

Subtype/ Type of infiltrate	Lymphocyte	Macrophage	Neutrophil	Plasma cells	Eosinophil
Type 2	8	1	7	2	4
Type 3	11	2	11	6	3
Type 4	11	2	12	6	6

TABLE 4: Distribution of intensity of eosinophilic infiltrate

Subtype/Intensity	1+	2+	3+	Total
Type 2	1		3	4
Type 3	2		1	3
Type 4	2	3	1	6

The clinical features were then correlated with the histological subtypes . State of drowsiness was found to be maximum in type 2 membrane (87.5%). Maximum cases with GCS less than 13 belonged to type 4 (scar inflammatory) membrane (30%) followed by both type 3 (haemorrhagic inflammatory) (16.67%) and type 2 (inflammatory) membranes (16.67%). [Fig 5]

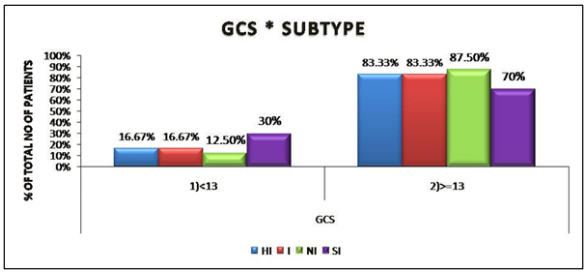


Figure 5: Correlation of GCS with subtype

Maximum mean GCS of 14.25 was found in type 1 (non inflammatory) membrane. [Fig 6] Minimum mean GCS of 13.1 was found in type 2 (inflammatory) membrane . [Fig 6]

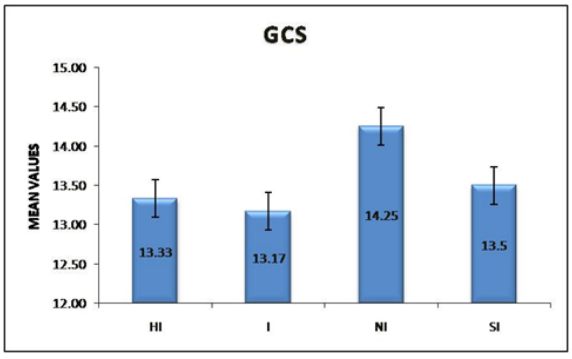


FIGURE 6: Mean GCS of various subtypes

Maximum mean thickness of subdural haemorrhage (6.88 cm) was found in type 4 (scar inflammatory) membrane. It was calculated by measuring the largest depth of hematoma on axial slices in centimetres. Then the mean of depths for all the four categories were compared.

COX-2 and VEGF positivity were analysed both with respect to the type of cells showing positivity as well as intensity and proportion of the cells staining. Both macrophages and fibroblasts showed the highest frequency of positivity followed by plasma cells, whereas neutrophils were consistently negative. COX 2 positivity analysis showed that the two types of membranes with highest positivity rates were type 4 (scar inflammatory, 91.67%) and type 3 (haemorrhagic inflammatory, 63.64%) [p value 0.003].[Figure 7]. VEGF positivity analysis similarly showed highest positivity in type 4 (100%) and type 3 (63.64%) membranes [p value 0.0001].[Figure 8] These two membranes also had the highest intensity and proportion scores for both COX2 and VEGF . Also noted was that 88.89% of non inflammatory membranes were negative for COX2 and 100% of these membranes were negative for VEGF. [Figure 7 and 8]

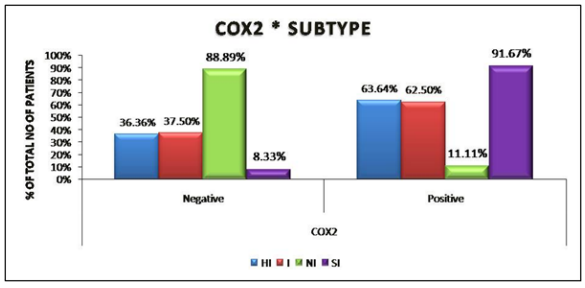


FIGURE 7: Correlation of COX2 with Subtype

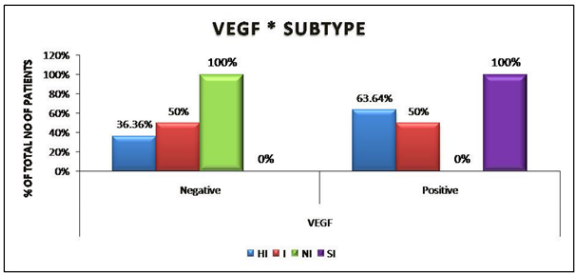


Figure 8: Correlation of VEGF with subtype

DISCUSSION

Subdural haemorrhage implies bleeding into the subdural space. When this bleeding turns into an organised unclotted hematoma covered by outer and inner membranes it is referred to as chronic subdural haemorrhage. Many theories have been proposed for the pathogenesis but none has been established. (Tanaka and Ohno, 2013) The earliest theory was given by Virchow who named it as 'pachymeningitis theory' according to which membrane forms due to inflammation because of bacterial meningitis . (Virchow et al,1857) This was followed by Trotter's theory according to which chronic subdural haemorrhage occurs due to trauma. (Trotter et al,1914) The most recent theory is the one given by Inglis in 1946. (Inglis et al,1946) He proposed the existence of 'dural border cells', with fibrogenic role. Injury to these cells leads to a propagated and continuous inflammatory response which in turn leads to membrane formation. The inflammatory cells produce various cytokines leading to neoangiogenesis.(Edlmann,2017) But the new vessels formed are leaky, have gap junctions and do not possess basement membrane. These vessels are responsible for continued leakage and hence growth of subdural haemorrhage. Finally the outer membrane is formed comprised of fibroblasts and collagen laid by the injured dural border cells, many inflammatory cells and numerous leaky capillaries. (Moskala et al ,2007; Hara et al ,2009; Shono et al,2001; Nanko et al ,2009; Sarkar et al,2002; Müller et al, 1990; Yamashima et al, 1983) The outer membrane produces various mediators like VEGF, tissue plasminogen activator, angiopoetin which play a crucial role in angiogenesis and fibrinolysis. (Ito et al, 1978; Hohenstein et al, 2005;. Nanko et al,2009; Murakami et al,2002; Nakagawa et al,2000) Due to angiogenesis more and more leaky vessels are produced leading to repeated leakage . Because of fibrinolysis, the hematoma does not clot and keeps on enlarging. These factors ultimately lead to recurrences. It was Suzuki et al who proposed that IL-6 and IL-8 are increased in the subdural space. (Suzuki et al,1998) These in turn induce COX2 which leads to increased production of PGE2. (Tsujii et al,1998) Ultimately PGE2 plays a role in synthesis of VEGF which through the PI3K-Akt pathway leads to angiogenesis.(Shono et al,2001; Funai et al,2011)

Chronic subdural haemorrhage is more common in males. (Bokka et al,2016) The same was found in our study [M:F- 3:1]. In 1993, Nagahori et al subdivided membranes into four histological patterns .(Nagahori et al, 1993) They correlated the duration from trauma to presentation with the histological subtype and found a temporal evolution from type 1 to type 4 membranes i.e. type 1 membranes belonged to the shortest duration hematomas followed in increasing order by type 2, 3 and 4 membranes respectively. In the study conducted by Nagahori et al , type 2 membranes were the commonest comprising 48% of total, followed by type 3 (32%), type 4 (23%) and least common were type 1(7%). (Nagahori et al, 1993) Few other studies have also similarly classified membranes like Min Ho Park et al found type 3 (39%) as the commonest and type 1 (6%) as the rarest subtype.(Park M et al,2015) We observed that type 4 was the commonest (30%), followed by type 3 membrane (27.5%). This may be due to delayed hospital consultation which is very common in the Indian scenario. The clinical features were then correlated with the histological subtypes. State of drowsiness was found to be maximum in type 2 membrane (87.5%). The type 2 membrane is inflammatory with maximum number of inflammatory cells, thereby producing the maximum amount of cytokines like IL-2, IFN-ϒ and TNF. This can be attributed as the reason behind the drowsiness. (AJ van de et al,2017) In a study conducted by Min Ho Park et al neurological deficit was associated maximum with type 4 (63%) and type 2 (56%) membranes. (Park M et al,2015) In our study, GCS less than 13 was found to be associated with type 4 (scar inflammatory) membrane (30%) followed by both type 2 and type 3 (inflammatory and haemorrhagic inflammatory) membrane (16.67% each). Maximum mean GCS of 14.25 was found in non inflammatory type of membrane. Minimum mean GCS of 13.1 was found in inflammatory type of membrane. This is similar to the findings of G S Gandhoke et al where they found that GCS less than 13 was exclusively associated with type 2 membrane.(Ito et al,1978) Maximum mean thickness of subdural haemorrhage (6.88) was found to be associated with type 4 (scar inflammatory) membrane. Similar result was seen by GS Gandhoke et al.(Ito et al,1978; Park M et al,2015) In this study type 4 membranes showed minimum Glasgow coma scores and maximum thickness on CT scans.

We performed detailed histological examination of the membranes noting the type of infiltrate and graded it based on density of infiltrate (Table 1 and 2). We observed eosinophils in 33% (13/40) of the cases. The density of eosinophils also varied from 1+ to 3+. Most of the high density (2+, 3+) cases belonged to type 4 membranes.(Table 4) In a study conducted by C Sarkar et al eosinophils were observed in 60% of cases (30/50) whereas Muller and Firsching found eosinophils in 100% of their cases.(Sarkar C et al,2002;Müller et al ,1990) There is no previous study where association of eosinophils with the membrane subtype was found. In our study, 46% of the cases which were positive for eosinophils belonged to type 4 membrane. The implication of this finding is in accordance with the findings of C Sarkar et al. They found a correlation between intensity of eosinophilic infiltrate with duration of hematoma, being highest in hematomas of more than three months duration. In our study too maximum eosinophils were found in type 4 membranes (scar inflammatory) which represent the membranes of longest duration hematomas as has been proven by Nagahori et al. (Nagahori et al,1993)

As already described above, Subdural haemorrhage is known to recur and the COX 2- PGE2 pathway leading to synthesis of VEGF and neoangiogenesis is postulated to be involved in the pathogenesis of repeated haemorrhages.(Schaumann et al ,2016) Previously M Hara et al have documented COX 2 expression by immunohistochemistry in the outer membranes of subdural haemorrhage. They also assessed levels of VEGF, PGE2, IL-6 and IL-8 in hematoma fluid and found them to be significantly higher than in serum. (Hara et al,2009) However, they did not study COX 2 positivity according to the histological subtype of the membrane. We analysed the same and found that the two types of membranes with highest positivity rates were type 4 (scar inflammatory, 91.67%) and type 3 (haemorrhagic inflammatory, 63.64%) [P value 0.003]. We also observed that macrophages were consistently positive for both COX-2 and VEGF which is in accordance with previous studies by Shono et al and Hara et al (Stroobandt et al,1995; Hara et al,2009). Also, VEGF positivity was noted in endothelial cells in our study, which is again in concordance with Shono et al who found increased VEGF mRNA in both macrophages and endothelial cells. However, neutrophils were consistently negative for COX-2 and VEGF. This is in contrast to Hohenstein et al who found correlation between total VEGF content and the number of neutrophils in hematoma fluid.(Hohenstein et al,2005) Also noted was that 88.89% of non inflammatory membranes were negative for COX2. This is an important negative finding as we can stratify the patients according to histology, to select the possible candidates for targeting COX2. We have noted in our study that type 4 membranes showed highest positivity rates for COX2, highest thickness on CT, highest positivity for VEGF and lowest GCS scores vis a vis type 1 membranes which showed least positivity for COX2, VEGF and highest GCS scores. Hence by simple histological subtyping, we can identify the potential targets for COX2 inhibitor and VEGF inhibitor therapy. COX 2 inhibitors can help reduce morbidity in the patients of subdural haemorrhage by preventing recurrences and hence preventing repeated surgeries. A prospective randomised phase II/III study: COXIBRAIN was undertaken to selectively inhibit COX 2 in subdural haemorrhage patients using Celecoxib (selective COX2 inhibitor) . Celecoxib was given in a dose of 200 mg twice a day till third postoperative day followed by 200 mg daily for 25 days and then was discontinued. (Schaumann et al ,2016) They found that of the ones who were treated, none had recurrence after two months follow up. Even after two years of follow up, only one patient showed recurrence. (Schaumann et al ,2016). COX 2 inhibitors do hold a promise in future for treating a subset of subdural haemorrhage patients. However, these drugs do have some contraindications to their use, so more systematic studies are required to prove their efficacy.

VEGF is responsible for neoangiogenesis and targeting it can be an alternative pharmacotherapy that can be offered to these patients. Few studies in the past have shown increased VEGF expression in the outer membranes. Weigel et al found a statistically significant correlation between VEGF concentrations in hematoma fluid and exudation rate (calculated on the basis of CT appearance). (Weigel et al ,2014). Vacquero et al have also detected immunohistochemical expression of VEGF in SDH membranes. (Vaquero et al,2002) Shashivadhanan et al , recently showed immunohistochemical expression of VEGF in 82% of the subdural haemorrhage patients undergoing craniotomy for an increased hematoma size, again, highlighting the role of VEGF in hematoma expansion. (Sundaravadhanan &Deb, 2017)We also found VEGF positivity in 100% of type 4 and 63.64% of type 3 membranes. Bevacizumab (VEGF inhibitor) has been recently found to be of great use in many cancers like breast, colon and non small cell carcinoma. It

is being used successfully in many ophthalmic disorders like diabetic retinopathy, wet ARMD and retinal vein occlusions. The rationale behind its use is inhibition of neoangiogenesis, which is also implicated in pathogenesis of SDH recurrences. Hence, it is a very promising drug which can be exploited to prevent SDH recurrences. A case reported by Suzuki et al where a patient with glioblastoma multiforme and associated subdural haemorrhage was treated by bevacizumab (VEGF inhibitor) as a routine protocol to treat recurrent glioma with edema. Surprisingly they found that after 6 weeks of therapy, SDH disappeared completely.(Suzuki et al, 2016)

To conclude, there is increased expression of COX2 and VEGF in chronic subdural haemorrhage. Both COX 2 and VEGF inhibitors hold promise of preventing recurrences in a subset of subdural haemorrhage patients and more studies should be directed towards these drugs. As shown by our study, minimum GCS scores, maximum CT thickness, maximum COX2 and VEGF positivity were found in type 4 membranes and minimum COX 2 and VEGF positivity was found in type 1 membranes. Hence, simple histologic typing can help in selecting the subset of patients which will be most benefitted with these therapies. Our study is the first study to highlight the need of histologic subtyping of chronic subdural haemorrhage to identify the candidates for pharmacotherapy with COX2 and VEGF inhibitors. This will surely benefit the patients in the long run by preventing repeat surgeries and reducing post-operative morbidity.

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