Original Article

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Biochemical and Cytological Comparison of Keratocystic Odontogenic Tumours to Nonkeratinising Odontogenic Cysts Fluid

Dentistry Section

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ABSTRACT

Aim: To evaluate the levels of albumin, prealbumin, total protein, inorganic phosphate and presence of keratinocytes in the cystic fluid for the diagnosis and appropriate treatment planning of keratocystic odontogenic tumours and other non keratinizing odontogenic cysts.

Materials and Methods: Fifteen keratocystic odontogenic tumour and 15 controls were studied. The cystic fluid was aspirated and analysed to determine the levels of albumin, prealbumin, total protein, inorganic phosphate and the presence of keratinocytes. The data collected was statistically evaluated using Mann Whitney U-Test and Student's t-test.

Results: A highly significant difference ($p<0.0001$) was seen when a comparison of Prealbumin, total protein, inorganic phosphate and presence of keratinocytes was made between keratocystic odontogenic tumour and non keratinizing odontogenic cysts. The presence of albumin also showed a significant difference ($p<0.01$).

Conclusion: A combined analysis of total protein, albumin, prealbumin, inorganic phosphorous and detection of epithelial squames may be used as a diagnostic adjunct in the preoperative diagnosis of keratocystic odontogenic tumour in a minimally invasive and highly accurate fashion.

Keywords: Cyst Fluids, Dentigerous cyst, Odontogenic keratocyst, Radicular cyst

INTRODUCTION

Keratocystic odontogenic tumour (KCOT)/ Odontogenic keratocyst (OKC) is a clinicopathologically distinct form of odontogenic cyst, known for its pathognomic microscopic features, aggressiveness and high recurrence rate [1]. The frequency of OKC has been reported to vary from 3% to 11% of odontogenic cysts [2]. OKC is one of the most aggressive odontogenic cysts owing to its relatively high recurrence rate and tendency to invade adjacent tissues [3]. This lesion is now categorized as an odontogenic tumour according to latest WHO recommendations because of its aggressiveness, infiltrative nature and mitotic activity of the epithelial cells which is greater than that of other odontogenic jaw cysts [4].

Conservative methods of treatment such as enucleation and marsupilization, consistently have produced less than optimal results in KCOT compared to that of nonkeratinising odontogenic cysts (NKOC) like dentigerous cyst (DC) and radicular cyst (RC). Hence various surgical modalities like curretage, peripheral ostectomy, osseous reconstruction with or without continuity defect were advised in an attempt to reduce the recurrence rate [5]. The propensity for KCOT to recur ranges from 25% to 60% [6]. KCOT may penetrate cortical bone and involve the surrounding soft tissues [7]. Since KCOT is more aggressive and tends to recur after surgical excision, it is important to differentiate it from other odontogenic cysts [8].

Attention has been drawn to the fluid as an integral part of a cyst and it was noted that the consistency of the contents of odontogenic cysts is variable ranging from a clear yellow liquid to a semi-solid cheese-like mass. Difficulties in the preoperative diagnosis of KCOT have enthused attempts to find a biochemical or immunological marker in aspirates of cyst fluid [9-11].

Studies have reported significant differences between the concentration of total protein, prealbumin, albumin as well as keratin and keratinocyte levels in cystic fluid of KCOTs and other odontogenic cysts [12]. Very few studies have been performed to determine the levels of inorganic phosphate and cytological aspects of the fluid for the preoperative diagnosis of KCOT.

Hence, the present study was planned to evaluate the levels of albumin, prealbumin, total protein, inorganic phosphate and presence of keratinocytes in the cystic fluid to diagnose and appropriately plan the treatment of KCOT and NKOC.

MATERIALS AND METHODS

Fifteen cases of KCOT and 15 controls of NKOC like DC and RC were studied from the Department of Oral Pathology and Microbiology, AB Shetty Memorial Institute of Dental Sciences, Mangalore, India. The cystic fluid was aspirated from the most prominent and fluctuant part of the swelling through an intact mucosa. One ml of the fluid was used for the estimation of albumin, prealbumin, total protein and inorganic phosphate. The remaining cystic fluid was placed for centrifugation at 1500rpm for 10 min. Following centrifugation, the cells which appeared at the base of the centrifuge tube was carefully removed using a pipette and was rapidly smeared. Three smears were prepared and were stained with Haematoxyline & Eosin stain (H&E), Papanicolaou (PAP) stain and May Grunweld Giemsa stain (MGG). After staining, the smears were examined under light microscopy for the presence of keratin and keratinocytes and were estimated on an arbitrary four point scale: (-) No keratin/keratinocytes, (+) Few keratin/keratinocytes, (++) Moderate number of keratin/keratinocytes, (+++) High number of keratin/keratinocytes.

Determination of total protein was done using direct Biuret method and inorganic phosphorous was determined using Phosphomolybdate methodology. Qualitative estimation of prealbumin and albumin was done by using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis and visualization of the prealbumin and albumin bands was made under standardized conditions of the intensity of Coomasie Brilliant Blue staining in trans-illuminated light on a scale: (-) no band, (+) faint band, (++)

moderate band, (+++) strong band and (++++) very strong band. The data collected was statistically evaluated using Mann Whitney U-Test and Student's t-test.

RESULTS

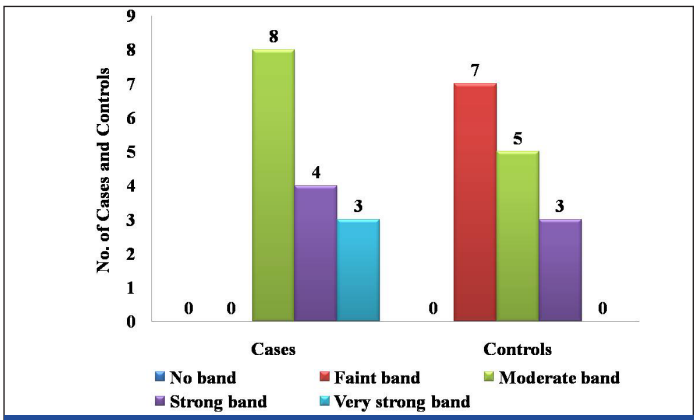
Fifteen cases of KCOT and 15 controls of NKOC were studied. The NKOC comprise of 4 DC and 11 RC. Albumin band was observed in all cases as well as controls [Table/Fig-1]. Out of 15 cases, 8 cases exhibited – a moderate band, 4 cases – a strong band and 3 cases – a very strong albumin band. Out of 15 controls, 7 controls exhibited – a faint band, 5 controls – a moderate band and 3 controls – a strong albumin band [Table/Fig-1,2].

The prealbumin band was present in all the cases where as it was absent in controls [Table/Fig-3]. Out of 15 cases, 6 cases exhibited a faint band, 5 cases – a moderate band, 2 cases – a strong band and 2 cases – a very strong prealbumin band [Table/Fig-2,3].

A comparison of total protein between cases and controls showed highly significant difference. The total protein content in the cases ranged from 2.11 to 6.85 gm/dl with a mean value of 3.984 gm/dl. The total protein content in the controls ranged from 6.1 to 11.2 gm/dl with a mean value of 8.541 gm/dl. A comparison of total protein between cases with inflammation and cases without inflammation also showed highly significant difference [Table/Fig-4]. Total protein content in 6 cases with inflammation showed a range of 4.6 – 6.85 gm/dl with a mean of 5.542 gm/dl and 9 cases without inflammation showed a range of 2.1 – 4.2 gm/dl with a mean of 2.946 gm/dl.

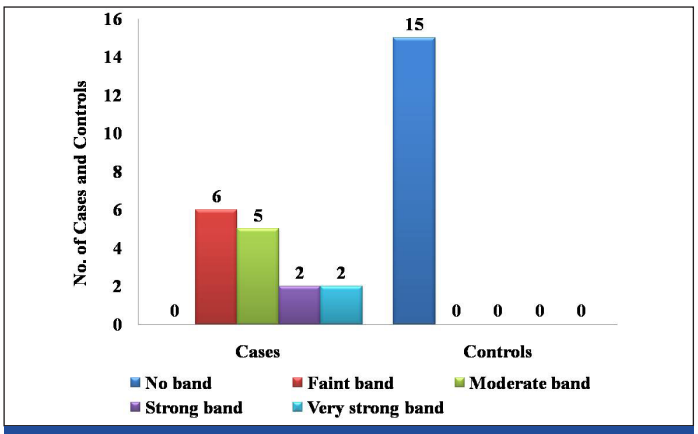
A highly significant difference was observed when a comparison of inorganic phosphorous was made between KCOT and NKOC [Table/Fig-5]. The inorganic phosphorous content in the cases ranged from 13.6 to 25.6 mg/dl with a mean value of 18.45787 mg/dl and the inorganic phosphorous content in the controls ranged from 4.3 to 21 mg/dl with a mean value of 10.85 mg/dl.

A comparison of cell count between cases and controls showed high significant difference. The epithelial cells were present in

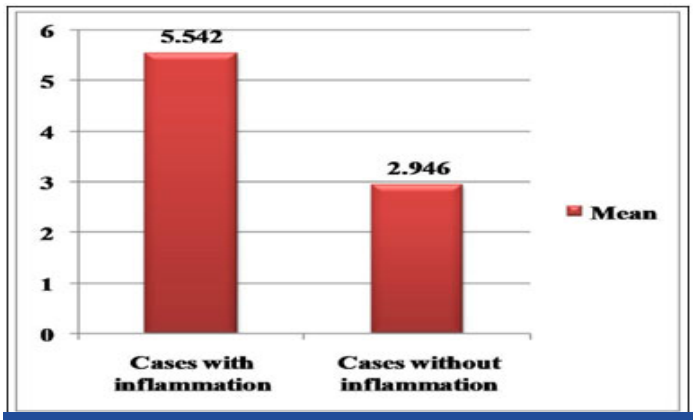


[Table/Fig-1]: Clustered bar chart comparing albumin between cases (KCOT) and controls (NKOC)

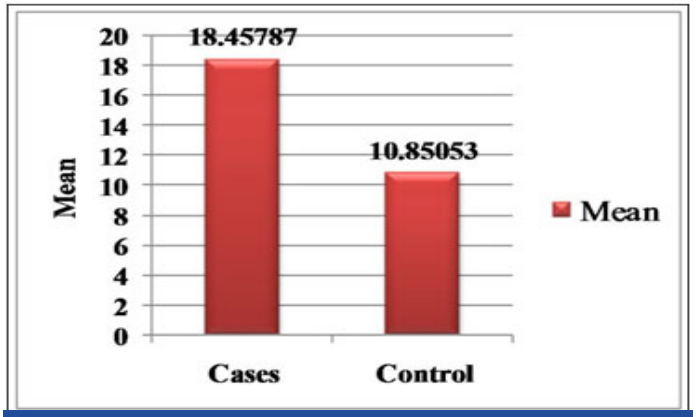
[Table/Fig-2]: Sodium Dodecyl Polyacrylamide Sulphate Gel showing prealbumin and albumin bands in KCOT as well as NKOC groups



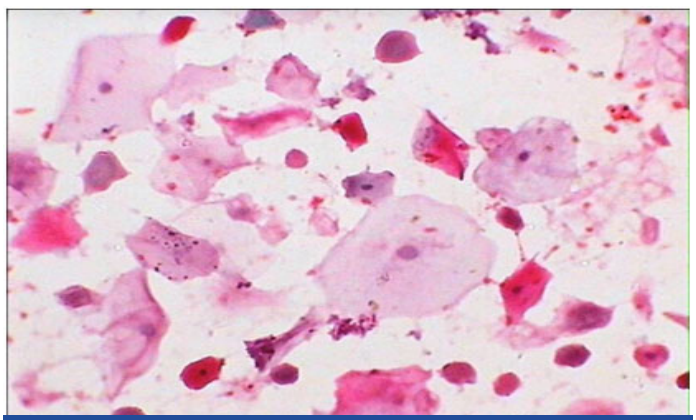
[Table/Fig-3]: Clustered bar chart comparing prealbumin bands between cases (KCOT) and controls (NKOC)



[Table/Fig-4]: Bar chart showing comparison of total protein between cases (KCOT) with inflammation and cases without inflammation



[Table/Fig-5]: Bar chart showing comparison of inorganic phosphorous between cases (KCOT) and controls (NKOC)



[Table/Fig-6]: Photomicrograph showing epithelial squames in the smears from KCOT (40X, Papanicolaou Stain)

93.333% of the cases [Table/Fig-6] and in 20% of the controls. The frequency of epithelial cells in KCOT and NKOC are shown in [Table/Fig-7,8] respectively.

DISCUSSION

In the present study an estimation of various parameters in the fluids of KCOT in comparison to other NKOCs showed striking differences. The difference in the composition of the cystic fluid of KCOTs, RC and DC supported the view that the pathogenesis of these lesions is different [13]. Levels of Albumin, prealbumin, total protein, inorganic phosphorous and epithelial cells in KCOT was compared with those of NKOCs.

Parameter	Category	Frequency	Percentage
Epithelial cells	-	1	6%
	+	4	26%
	++	7	46%
	+++	3	20%

[Table/Fig-7]: Frequency table showing epithelial cells in the cases (KCOT)

Parameter	Category	Frequency	Percentage
Epithelial cells	-	12	80%
	+	3	20%
	++	0	0%
	+++	0	0%

[Table/Fig-8]: Frequency table showing epithelial cells in the controls (NKOC)

A significant difference ($p<0.01$) was seen when a comparison of albumin was made between KCOT and NKOCs [Table/Fig-1]. Electrophoresis showed the presence of an albumin band in cystic aspirates from both the study and control groups [Table/Fig-2]. When compared to the control group, the study group showed higher albumin levels and within the control group, DCs contained more amount of albumin than RCs. Shear found that RCs contain lower levels of albumin and higher levels of β and γ globulin. The presence of inflammatory changes in the cyst wall and abundant inflammatory cells in cyst fluid make it likely that the proteins are derived as inflammatory exudates [14].

Toller concluded that the fluid of OKC composed of predominately albumin with relatively small quantities of immunoglobulins [15]. The wall of OKC is characteristically free from signs of inflammation and thus the formation of inflammatory exudates and local synthesis of immunoglobulins would be expected to be low [16]. Even if these did occur to any extent, the continuous layer of keratinized epithelium lining the cysts forms a less readily penetrable barrier than the frequently discontinuous, non-keratinized epithelium lining RCs and DCs, and thus lower levels of proteins would accumulate in the cystic fluid [17]. In a study by Southgate et al., cystic fluid from OKCs was analysed. They found that the major protein fraction with mobility anodal to albumin on electrophoresis was shown not to be albumin or prealbumin but a non-serum protein [10].

Smith et al., aspirated cystic fluid from 18 OKCs and found a lower level of albumin, globulins and total protein (4.70 ± 0.73gm per 100ml) when compared to RCs and DCs. The higher proportion of albumin relative to total globulins in OKC was reflected in the higher albumin: globulin ratio observed. They suggested that in a cystic fluid of 100µl; a qualitative protein electrophoresis, protein analysis as well as smears can be prepared. They reported that in all cases, an accurate diagnosis of OKC could be made when a protein analysis and smears which demonstrate epithelial squames were combined together [18,19].

Toller and Holborow also found higher ratios of Immunoglobulin levels in cystic fluid to autologous serum immunoglobulin levels [20]. They found higher levels of IgA, IgG, and IgM levels in cystic fluid than in the sera of the same patients. Higher levels of IgA were seen as compared to IgG, and IgM in cystic fluid. They also suggested that these proteins are transported to cystic fluid by immunoglobulin producing cells itself [20]. Stosiek & Klammt had also measured levels of IgG, IgA and IgM in cystic fluid as well as serum of the same patients. They found that their levels in cystic fluid are 1.3 to 1.5 times higher than that found in normal human serum [21]. Hamidreza et al., found higher levels of albumin in radicular cyst as compared to other odontogenic cysts. They also observed a higher concentration of α1 and β globulin in radicular cyst as compared to other odontogenic cysts [22].

A highly significant difference ($p<0.0001$) was seen when a comparison of the prealbumin band was made between KCOT and NKOCs [Table/Fig-3]. The present study showed that the prealbumin band was present in all KCOT whereas it was absent in NKOCs. Most of the KCOTs showed a faint to moderate prealbumin band [Table/Fig-2]. Browne concluded that the pre albumin band was more diffuse in the cystic fluid than in the autologous serum and in most instances was indistinct. There was a distinct prealbumin fraction in 9 fluids, all of them being OKCs [12]. The prealbumin is present in traces in RCs and DCs and is not always prominent in the electrophoretogram of cystic fluids [23].

Toller found that the cystic fluid of OKCs contain a lower concentration of soluble protein and may contain a major protein fraction with prealbumin mobility on electrophoresis. He found this prealbumin band in seven of seventeen OKCs examined. The band was not seen in a series of seventy-one non keratinising dental cysts. The presence of the band has been suggested to be of value in the diagnosis of the OKCs [15]. Prealbumin bands can be observed in inflamed as well as non-inflamed OKCs where as the other factors like total protein and epithelial squames tend to alter in the presence of inflammation. A prealbumin band can be diagnostic in cases of KCOT with or without inflammation. The molecular weight of prealbumin is less than albumin [24]. This might be a reason for the greater amount of prealbumin in the cystic fluids of KCOT.

A highly significant difference ($p<0.0001$) was observed when the total soluble protein in aspirates of KCOTs was compared with the aspirates of NKOCs. Concentration of total soluble protein in aspirates of KCOTs ranged from 2.11 to 6.85 gm/dl and in the NKOCs it ranged from 6.1 to 11.2 gm/dl.

Toller showed that fluids from keratinizing cysts had soluble protein levels below 3.5 gm/100 ml (mean 2.2 gm/100ml) but the values for non-keratinising cysts were in the range 5.0-11.0 gm/100ml with a mean of 7.1 gm/100ml. Toller postulated that a protein level of less than 4.0 gm/100ml indicated a diagnosis of OKC. A value of over 5.0 gm/100ml, however would suggest a RC, DC, fissural cyst, or even an ameloblastoma [15,19]. His view was that fully keratinized linings were impervious to all proteins whereas the epithelial lining of RC or DC would at least slowly transmit the smaller proteins. In the presence of a fairly pronounced inflammatory reaction in an OKC epithelial lining, the degree of keratinization in these areas would be altered. This change is likely to increase the permeability of the lining and result in a higher soluble protein level in the cystic fluid than that in the non inflamed keratinizing cysts [15,19]. The present study also showed an increased concentration of total protein in KCOTs which might be due to presence of inflammation in the cyst wall [Table/Fig-4]. Skaug and Hofstad observed an evidence for the view that the bulk of the cyst fluid proteins are derived from the plasma [23]. Browne in his study also concluded that the soluble protein of the cyst fluid resembles a transudate of serum, with a very small local production of immunoglobulins, even if the cyst becomes infected [13].

A highly significant difference ($p<0.0001$) was observed when the inorganic phosphorous level in aspirates of KCOTs was compared with the level seen in NKOCs [Table/Fig-5]. The concentration of inorganic phosphorous in KCOT ranged from 13.6 to 25.6 mg/dl and in the NKOCs it ranged from 4.3 to 21 mg/dl. The inorganic phosphate content of the cystic fluid of DC was found to be least when compared to KCOT and RC. These findings are consistent with a study done by Browne et al., in which they found a high

incidence of crystalline calcium phosphates, hydroxyapatite and whitlockite and inorganic phosphates in the aspirated fluid of 38% of KCOT when compared with 10% of RC and 0% of DC. They concluded that the higher levels of these ions in the cystic fluid may possibly be responsible for the higher frequency of deposits in their walls [19,25].

Browne also found greater prevalence of mineralization in the capsules of OKC compared to RC or DC [26]. Increased concentration of inorganic phosphate may be responsible for the mineral deposits seen in the walls of OKCs. Watanabe et al reported a calculus-like deposit in two cases of periapical cysts with long-standing sinus tracts [27].

A highly significant difference ($p<0.0001$) was seen when a comparison of the number of epithelial cells was made in the fluid aspirates of KCOT [Table/Fig-6] and NKOCs. The epithelial cells were present in 93.333% of KCOTs where as it was seen in only 20% of NKOCs. Kramer suggested that a preoperative diagnosis of OKC might be made by aspirating cystic fluid and demonstrating keratinized squames in the stained film [19,28]. Kramer and Toller reported on the combined use of epithelial cells and protein estimation of the cystic fluid in the preoperative diagnosis of cysts. They found that examination of the smears achieved comparable diagnostic accuracy to that achieved by use of the protein estimation [19,29]. Voorsmit reported a diagnostic accuracy of 100% when the combined techniques were used, using a total protein level in the cyst fluid of less than 4.8gm/100ml as an indication that the lesion was an OKC [19,30]. RM Browne suggested that there was a high incidence of epithelial cells in OKCs and not in other lesions. The epithelial cells were present in only 11.4% of RCs and 6.7% of DCs but were present in 83.3% of OKCs [12]. The marked differences in the composition of the fluids from OKCs compared with RC and DC lend no support to the views that the OKCs arise by changes occurring in previously existing RCs or DCs. This finding is supported by studies done by Browne [12].

In addition to helping the understanding of the pathogenesis of the odontogenic cysts, these differences in the fluid content can aid in establishing a correct preoperative diagnosis. The withdrawal of fluid for protein analysis as a preoperative procedure has been previously advocated by Browne and Toller [12,31]. The results presented here confirms this view.

LIMITATIONS

There were a few limitations in the present study. The presence of inflammation can change the parakeratinized lining of KCOT to a non keratinized lining thus increasing the chances of obtaining nucleated cells in the cystic aspirate. Although none of the methods gave complete accuracy, the number of incorrect diagnosis were reduced if smears were stained by two or three methods (H&E, PAP or MGG) along with biochemical estimations performed on each case. Even though the cystic fluid is thick and cannot be aspirated in higher quantities, the smears can be prepared, since it needs very less amount of fluid [19]. The findings from the present study may help in differentiating KCOT from NKOCs but it may not be of much help in differentiation of orthokeranized odontogenic cyst from KCOT.

Because of more troublesome clinical behaviour of KCOT, the preoperative diagnosis is essential for its proper management. Fluid containing low protein, consisting predominately of albumin comparatively high inorganic phosphorous, a prealbumin band on electrophoresis and epithelial squames is characteristic of KCOT. As this lesion may resemble either a RC or DC clinically and radiographically, this procedure is most valuable [32]. It is important to record all the parameters to arrive at the correct diagnosis, the use of only one may lead to an incorrect diagnosis.

Despite the limitations of this study, a marked difference was seen in the composition of the cystic fluids from KCOTs compared with those from RCs and DCs. The present study showed that the presence of epithelial cells, decrease total protein, a distinct prealbumin band, increased inorganic phosphorous as well as albumin in the cyst fluid are diagnostic of KCOT. This study also provides informative values in appropriately planning the treatment of KCOT and other odontogenic cysts, thus providing a relatively non-invasive technique prior to surgery.

CONCLUSION

With a few limitations, the present study indicates that a combined analysis of total protein, albumin, prealbumin, inorganic phosphorous and detection of epithelial squames may be used as a diagnostic adjunct in the preoperative diagnosis of KCOT in a minimally invasive and highly accurate fashion.

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