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Determination of Proteins and Cholesterol Levels in the Fluid of Jaw Cystic Lesions in Patients Attending Khartoum Teaching Dental Hospital

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Abstract

This study determined and compared levels of proteins and cholesterol in the fluid of Jaw cystic lesions in patients attending Khartoum Teaching Dental Hospital.

Methods: An observational comparative hospital based study was done in a total of 45 patients cystic fluids. Patient's age ranged from 9 to 47 years. Colorimetric methods were used and data was analyzed by T-test.

Results: Lowest values of total protein were recorded in keratocystic odontogenic tumour (KCOT); mean value being approximately (2.9 g/dl±1.5). In radicular cysts; highest value of total protein were recorded with a mean of (8.5 g/dl±2.0). Highest value of total cholesterol was also recorded in radicular cysts with a mean of (189 mg/dl±50).

Conclusion: The study confirmed that the fluid of KCOT has a low protein content of less than 4g/dl. There is significant difference in the mean of total proteins between KCOT and other cysts. No significant differences were found between a mean of total protein and a mean of total cholesterol in KCOT and cystic ameloblastoma.

Keywords: Jaw cystic lesions; Proteins; Cholesterol; Keratocystic odontogenic tumour; Dentigerous cyst; Cysic *ameloblastoma*

Abbreviations: KCOT: Keratocystic Odontogenic Tumor; KTDH: Khartoum Teaching Dental Hospital; SPSS: Statistical Package for Social Sciences

Introduction

Cystic lesions of the jaws have been recognized as clinical problems since very long time. The jaw cysts of

odontogenic origin are the most common cystic lesions that affect the human skeleton [1,2]. Kramer has defined a cyst as a pathological cavity having fluid, semi fluid or gaseous contents and which is not created by the accumulation of pus. Most cysts, but not all, are lined by epithelium [3,4].

Cystic lesions of the jaws may be epithelial or non-epithelial, odontogenic or non-odontogenic, developmental or inflammatory in origin. In a recent study by Bodner et al, the distribution of jaw cysts according to diagnosis in a general population was radicular cysts 55%, dentigerous cysts 17%, nasopalatine duct cysts 12%, keratocysts 11%, then other types of cysts etc. [4]. In the last WHO classification in 2005 the odontogenic keratocyst was reclassified from cyst to tumor. The new term: keratocystic odontogenic tumor (KCOT) [5].

The purpose of the present study was to determine levels of proteins and cholesterol in the fluid of jaw cystic lesions in a group of patients attending Khartoum Teaching Dental Hospital (KTDH); and to compare these levels in the fluid of KCOT and other types of jaw cystic lesions. Also, the study aimed to verify the estimation of proteins and cholesterol levels in the fluid of the jaw cystic lesions as a simple method to aid in the pre-operative diagnosis.

Material and Methods

This is an Observational comparative hospital based study, carried out on Patients with cystic lesions in the jaws attending Oral and Maxillofacial Surgery Units in KTDH, during May 2009 to March 2012. Fluid aspirated from 45 jaw cystic lesions, as a nine

samples from KCOT, 9 samples from dentigerous cysts, 9 samples from radicular cysts, 7 samples from nasopalatine cysts and 11 samples from cystic ameloblastoma.

The cystic lesions were diagnosed from their clinical, radiological and histological features. The fluid was aseptically aspirated from the cyst without blood contamination of enough volume (at least 2ml), and it was kept at 2-8 °C. In the laboratory, the Fluid was centrifuged at approximately 4000rev/min for 5 minutes to separate cellular material from other particulate materials. The cellular material was pipetted into a labeled test tube, and thoroughly mixed by shaking the tube and then allowed to set for 10 minutes at room temperature (16-25 °C). Reading the absorbance (A) of the standard and sample against the blank: at 545nm for total protein, at 630nm for albumin and at 500nm for cholesterol (Figure 1) The concentration of total protein, cholesterol and albumin is obtained automatically in the spectrophotometer by the following formulae:

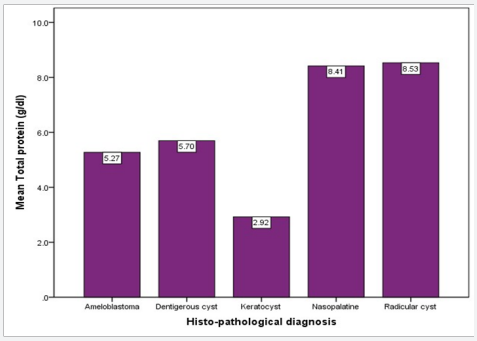

Figure 1: Mean of total protein in all samples.

A sample / A standard * C standard = C sample (by g/l for total protein and mg/dl for cholesterol).

Formula for calculation of albumin:

A sample / A standard * 5 (standard concentration) = g/dl albumin in the sample.

Bacterial growth in each cystic fluid was assessed by culture.

Methods description and materials used

1. Total protein estimation by Biuret method: Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrometry.

2. Quantitative determination of albumin: Albumin in the presence of bromocresol green at a slightly acid pH produces a colour change of the indicator from yellow-green to green-blue. The intensity of the colour formed is proportional to the albumin concentration in the sample.

3. Cholesterol estimation by chemical method (Cholesterol oxidase / peroxidase): Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

Cholesterol ester + H_2O Cholesterol esterase cholesterol + Fatty acid

Cholesterol + 1/2 O2 + H_2O Cholesterol oxidase cholestenone + H_2O

2 H_2O + 4-Aminoantipyrine + phenol Peroxidase Quinoneimine + 4 H_2O

4. Measuring range (detection and linearity limits):

For total protein: from 4.6g/l up to 150g/l (0.46g/dl up to 15g/dl)

For albumin: from 0.04g/dl up to 6g/dl

For cholesterol: from 0.3mg/dl up to 1000mg/dl

a. Reagent and standard for estimation of the total protein:

Reagent: copper (II) acetate 6mmol/l, potassium iodide 12mmol/l, sodium hydroxide 1.15mol /l, detergent.

5. Protein standard: Biuret reagent

a. Reagent and standard for quantitative determination of albumin:

Reagent: Bromocresol green, pH=4.2

Standard bovine Albumin: 5g/dl albumin aqueous primary standard.

b. Reagent and standard for estimation of Cholesterol:

Reagent: pipes 35mmol/l, sodium cholate 0.5mmol/l, phenol 28mmol/l, cholesterol esterase 0.1U/ml, peroxidase 0.8U/ml, 4-aminoantipyrine 0.5mmol/l, pH 7.0

Cholesterol standard: Cholesterol 200mg/dl (5,18mmol/l). Aqueous primary standard

a. Equipments for estimation of total protein, albumin and cholesterol:

b. Disposable syringe (5ml) for aspiration of the cystic fluid (22 gauges).

c. Automatic variable pipette.

d. Spectrophotometer (Biosystem BTS-310, made in spain) able to read from 500nm up to 630nm.

e. Centrifugator (made in Germany, by Hettich Zentrifugen Company), model EBA 20.

f. Labeled test tube.

Statistical method

The data were analyzed by the statistical package for social sciences (SPSS) version 19. They were tested for normality and no significant deviation from normality was observed. Because the data were found to be symmetrical, the standard descriptive statistics tools such as mean and standard deviation were used.

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The 95% confidence interval for the mean of proteins and total cholesterol were established for different cysts. The level (on average) of proteins and total cholesterol were tested for comparison between keratocysts and other cysts by the T-test. This was done at 0.05 significant levels. The data were presented graphically and in tabular format.

Results

Table 1: The mean level of total protein, albumin, globulins and cholesterol in all samples.

	Ameloblastoma		Dentigerous cyst		Keratocyst		Nasopalatine cyst		Radicular cyst	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Total Protein (g/dl)	5.3±1.2	3.5-7.9	5.7±0,9	4.7-7.6	2.9±1.5	1.2-4.9	8.4±2.9	5.6-13.9	8.5±2	6.1-11.7
Albumin (g/dl)	3.2±0.8	2.0-5.0	2.9±0.9	1.2-3.9	2.0±1.3	0.3-4.2	4.8±1.2	3.5-7.2	4.2±1.6	2.4-7.7
Globulins (g/dl)	2.0±0.7	1.1-2.9	2.8±0.8	1.7-4	0.9±0.7	0.2-2	3.6±1.7	3.5-7.2	4.4±1.3	2.4-7.7
Cholesterol (mg/dl)	88±23	62-133	105±42	50-167	135±87	25-260	155±68	88-288	189±50	134-310
Abumin/ Globulins ratio	1.71±0.57	1.07-2.75	1.3±0.64	0.3-2.29	4.34±6.44	0.3-21.0	1.51±0.63	1.0 - 2.5	1.06±0.56	0.46-2.14

Table 2: The mean level of total protein, albumin, globulins and cholesterol in sterile samples in comparison to contaminated samples.

	Ameloblastoma		Dentigerous cyst		Keratocyst		Nasopalatine cyst		Radicular cyst	
	Sterile	contaminated	Sterile	contaminated	Sterile	contaminated	Sterile	contaminated	sterile	Contaminated
Total Protein (g/dl)	5.2±1.4	5.5±0.1	5.2±0.5	6.3±1.1	2.5±1.4	4.6±0.5	7.2±1.4	10.1±3.7	8.2±2.2	8.9±1.9
Albumin (g/dl)	3.2±0.8	3.4±0.6	2.7±1.1	3.2±0.6	21.7±1.4	2.9±0.0	4.3±0.2	5.4±1.9	4.5±2.0	3.7±1.1
Globulin (mg/dl)	2.0±0.7	2.2±0.8	2.5±0.7	301±1.0	0.7±0.6	1.7±0.5	2.9±1.3	4.6±1.9	3.7±1.0	5.2±1.2
±1.0	0.7±0.6	1.7±0.5	2.9±1.3	4.6±1.9	3.7±1.0	5.2±1.2				
Cholesterol (mg/dl)	86.8±25.5	91.0±15.6	95.6 ±45.9	116.8 ±40.8	130.7 ±95.6	151.5 ±67.2	190.8 ±69.9	106.7 ±19.6	198.6 ±67.1	178.0±20.7
Abumin/ Globulins ratio	1.7±0.5	1.7 ± 0.9	1.2±0.8	1.5±0.5	5.1±7.3	1.8±0.5	1.7±0.8	1.2 ± 0.1	1.3±0.7	0.7 ± 0.2

Levels of total protein, albumin, globulin and cholesterol were presented in Table 1. The mean level of total protein, albumin, globulins and cholesterol in sterile and contaminated samples were presented in Table 2 Sterile and contaminated specimens: In 9 samples of KCOT, 7 samples (78%) were sterile, while 2 samples (22%) were infected. Among 9 samples of dentigerous cysts, 5 samples (56%) were sterile while 4 samples (44%) showed bacterial growth. Of the 11 samples of cystic ameloblastoma, 9 were sterile (82%) while 2 samples (18%) showed bacterial growth. Highest percentage of bacterial growth was observed in the samples of radicular and nasopalatine cysts. Among 9 samples of radicular cysts, 5 samples (56%) were sterile, while in 7 samples of nasopalatine cysts, 4 samples (57%) were sterile and remaining were infected.

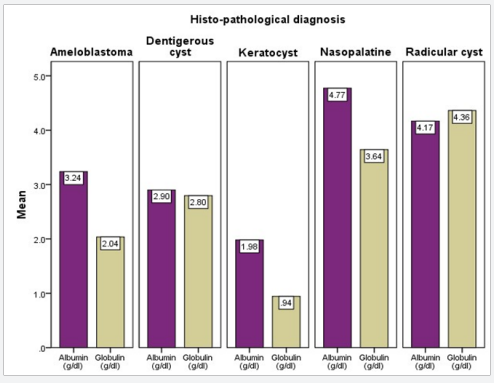

Figure 2: Mean of albumin versus mean of globulins in all sample.

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Discussion

Total protein concentration

The results of this study correlates with those of other researchers who stated the lowest concentration of soluble protein in fluid of keratocysts in comparison to other cysts [6-10]. In this study the lowest concentration was recorded in the fluid of KCOT (mean level was 2.9g/dl±1.5). The results also showed that infected samples of KCOT are associated with higher concentration of total protein. The highest total protein concentration was found in radicular cysts, the mean being 8.5 g/dl±2.0; thus reflecting the characteristic of inflammatory exudates, which is due to inflammatory radicular cysts. This is corresponding to the result of Skaug, [11] Douglas et al. [8], and Smith et al [9]. The study proved that the protein level of dentigerous cysts is similar to that in the serum [6], the mean level was 5.4 g/dl±0.9. While the fluid of nasopalatine cysts showed relative high concentration of total protein with a mean of 8.4g/dl±2.9, this is attributed to upper respiratory tract infection. The results of this study revealed that the mean of total protein for cystic ameloblastoma was 5.3 g/dl±1.2; this is similar to the study of Browne [6]. The mean of total protein concentrations of KCOT fluid was significantly lower than those in radicular, dentigerous, and nasopalatine cysts. This is similar to the results of Browne [6], Smith [9], and Douglas et al. [8]. Although according to this study, the difference was not significant between KCOT and cystic ameloblastoma (p value=0.257), these results are not corresponding to the results of Browne [6].

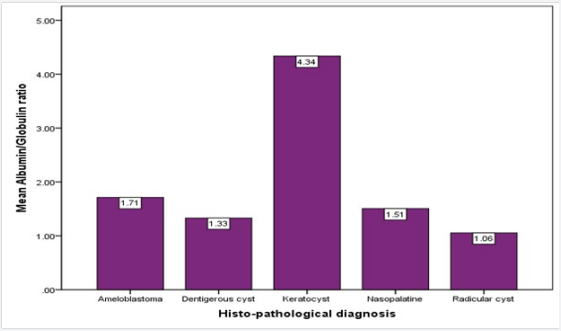

Figure 3: Mean of albumin/globulins ratio in all samples.

Albumin, globulins concentrations and albumin/globulin ratio

In the present study the highest value of albumin in comparison to its own globulins concentration were recorded in KCOT, with a mean of 2.0 g/dl±1.3. In this study high albumin percentage was (67.8%) is close to the result of (70%) which has been reported by Browne [6]. The results showed lowest recorded value of globulins in KCOT, with a mean of 0.9 g/dl±0.7. The highest value was observed in radicular cyst. Browne [6] found that radicular cysts contained high amount of globulins, in contrast to keratocysts which contained low amount of it. Smith & Browne [10] suggested that the higher ratio of albumin to globulins in keratocysts can be used as a preoperative diagnostic marker for this lesion [11]. This study confirmed the suggestion of Smith and Browne and it also revealed the highest values of albumin/globulins ratio in KCOT with a mean of 4.34±6.44 [7,12].

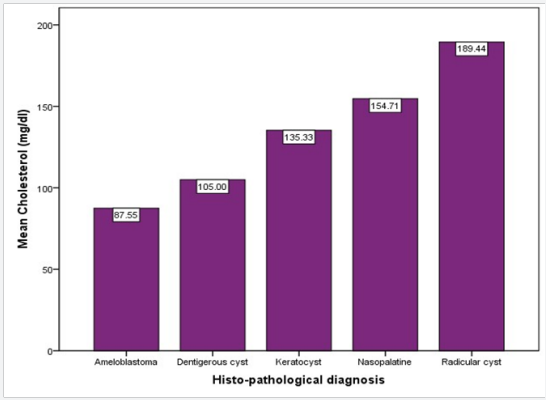

Figure 4: Mean of total cholesterol in all samples.

Total cholesterol concentration

The results of this study showed the highest values of total cholesterol were recorded in radicular cysts with a mean of 189 mg/dl±50, this is due to inflammatory process of radicular cysts and this is not corresponding to the result of Skaug [12]. In cystic ameloblastoma, the lowest value was observed. Finally the study showed no significant difference in the mean of total cholesterol between KCOT and other cysts in addition to cystic ameloblastoma [13].

Figure 5: Ethical approval.

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Conclusion

The present study confirmed that the fluid from KCOT has a low protein content of less than 4g/dl (mean

2.9g/dl), this will choose the appropriate method of treatment in order to reduce recurrent rate and help to follow the patients for longer period. Results showed high value of proteins and total cholesterol in radicular cyst in comparison to other cysts. They confirmed the suggestion that higher ratio of albumin to globulins in KCOT can be used as a preoperative diagnostic marker for this lesion. They also revealed that albumin concentration is higher in comparison to globulins concentration. Contaminated samples showed high value of proteins and total cholesterol concentration. There is significant difference in the mean of total protein between KCOT and other jaw cystic lesions.

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Conflict of Interest

The authors have no conflict of interest to declare. The authors did not receive any funding for this work.

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