IMMUNOHISTOCHEMICALSTUDY FOR BCL-2 IN ODONTOGENIC KERATOCYST

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This dissertation submitted for fulfill the requirements for the degree of Master of Science in oral pathology

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University of Benghazi **Faculty Of Dentistry**

Department of Oral Medicine
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بسم الله الرحمن الرحيم

" قالوا سبحانك لا علم لنا الاماعلمتنا ,انك انت العليم الحكيم"

صدق الله العظيم

سورة البقرة آية رقم 32

DEDICATION

I dedicate this work to my parentsand my family for supporting me throughout my life.

Acknowledgment

Firstly, thanks to **ALLAH** for the successful completing of this work.

Secondly, I wish to express deep sense of gratitude to my supervisor. **Prof. DrAzzam A. Sultan** for his suggestion, support and guidance throughout the period of this work. Also I would like to thank **prof. Dr Ali M. Elmurtadi**for assistance and cooperation to fulfill my work.

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Candidate

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
Aas	Amino acyl residues
ABC	Avidin-Biotin Complex technique
AOTs	Adenomatoidodontogenictumours
BMP	Bone Morphogenetic protein
CEOT	Calcifying epithelial odontogenic tumor
CDKIs	Cyclin-dependent kinase inhibitors
DAB	Diamino-BenzidineSoluation
EGFR	Epidermal growth factor receptor
GG S	Gorlin –Goltz syndrome
GLI1	Gli-family zinc-finger transcription factors
IHC	Immunohistochemical study
KCOT	Keratocysticodontogenic tumor (KCOTs),
MOMP	Mitochondrial outer membrane permeabilization
NBCCS	Nevoid basal cell carcinoma syndrome
OKC	Odontogenickeratocysts
OOC	Orthokeratinizedodontogenickeratocyst
PCNA	Proliferating cell nuclear antigen
PTCH	Patched 1 protein
PCNA	Proliferating cell nuclear antigen
SHH	Sonic Hedgehog protein
P53	Phosphoprotein 53
Ki-67	Ki-refer to the city of Kiel where the antibody was produced in and 67 is refer to number of original clone
Bcl-2	B-cell lymphoma 2
SMO	Smoothened proteins
WHO	World Health Organization

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Abstract

Introduction: The odontogenickeratocyst (OKC) is a relatively uncommon lesion which has much interest because of its unusual growth pattern and tendency to recur. The majority of odontogenickeratocysts arise sporadically and present as solitary lesions unless they are associated with nevoid basal cell carcinoma syndrome,although occurs in any part of jaws the majority of themfound in the mandible; most commonly in the posterior body and ascending ramus. OdontogenicKeratocycts present an aggressive clinical course with a marked trendtoward recurrence as compared with dentigerous cysts, differences in the clinical behavior of cysts may be associated with apoptosis in the lining epithelium.

Aim of Study:

1-To analyze the clincohistopathological features of the OdontogenicKeratocyst cases.

2- To study the immunoexpression ofBcl-2 inOdontogenicKeratocyst.

Materials and Methods:

A descriptive case- series study. Sample size were twenty casesdiagnosed OKC ranged from 18 -65 years M: F ratio 1.9: 1,the personal data such as age, sexsite, residence, nationality of the patients were noted from the patients recordes,the samples were taken from wax blocks ,cut and stained,four microns thick sections were cut and stained with Hematoxylin and Eosin (H & E) then stained by immune marker Bcl2 in the oral pathology department at university of Alexandria- Egypt to study immune reactions, finally the data were collected and analyzedusing statistical package social science (SPSS) version 17.

Results:

The histopathological appearance of OKCS , characterized by thin stratified squamous epithelium in the majority of cases. Most of OKCs showed a palisaded basal cell layer. The surface layer of cyst lining wereparakeratinized and only few were orthokeratinized,cyst wall contained the following:
Inflammatory cell were (75%) of cases,Satellite cysts were(40%) of cases, epithelial residues in (40%) of cases,Kerattinesquames in (60%) of cases.
Regarding immune histochemical finding, theBcl-2 expression positive cells was 75% of the lesions were classified into three grades weak , moderate and intense reactions in 20 %, 33.3 % & 46.6 % respectively.

Conclusion:

Inconclusion OKCs were solitary, the majority of OKC seems to be biologically aggressive and should be classified as a tumour rather than a cyst. Because the majority of OKCs were with high proliferative activity and characterized by higher expression of Bcl-2 in basal cell epithelium, probably that lesions are developmental cysts with some neoplastic properties.
We recommend to complete the medical files in the archive of oral pathology department to include all data such as onset of symptom, duration of lesions , if there is any associateddiseases and the report of radiography.

Chapter 1

INTRODUCTION

1. Introduction

The odontogenic keratocyst (OKC) Is a relatively uncommon lesion which has much interest because of its unusual growth pattern and tendency to recur [(1)]. The majority of odontogenic keratocysts arise sporadically and present as solitary lesions unless they are associated with nevoid basal cell carcinoma syndrome, They may occur in any part of jaws with the majority of lesions occurring in the mandible; most commonly in the third molar region and ascending ramus[(1)].

Odontogenic Keratocyst occur over a wide age range, but there is pronounced peak incidence in the second and third decades with a second smaller peak in the fifth decade. The cysts are more common in males than females[(1)],multiple cysts are associated with the naevoid basal cell carcinoma syndrome (NBCC syndrome or Gorlin-Goltz syndrome), inherited as an autosomal dominant trait with variable expressivity.

The syndrome is uncommon [(1,2,3)] ,contrast to radicular and dentigerous cysts, OKC tend to expand in anantero-posterior direction and can reach large sizes without causing gross bony expansion they are often discovered on routine radiographic examination[(3)],recurrence rates vary in different reported series from around 3 % to about 60 %[(4)], It is likely that the rate is decreasing with improved management following recognition of this problem [(4)].

The diagnosis of Odontogenic Keratocycts (OKCs) including thin epithelium (6-10 cell layers), refractile, parakeratotic lining, epithelial budding and daughter cysts, characteristic microscopic features lost when inflamed[(4)], radiographically, keratocysts appear as well-defined radiolucencies that may be unilocular or multilocular many present in apparent dentigerous relationship associated with unerupted third molars but the crowns of such teeth are usually

separated from the cyst cavity, the pericoronal tissues being continuous with the cyst capsule. Keratocysts may also present as developmental lateral periodontal cysts [4].

Odontogenic Keratocycts present an aggressive clinical course with a marked trend toward recurrence as compared with dentigerous cysts,there are two types of Odontogenic Keratocyst parakeratotic and orthokeratotic, the parakeratotic type forms 85 to 95% of all Odontogenic Keratocysts; the balance is made up of the orthokeratinzed variant [4].

Histologic distinction between the para and orthokeratinzed variants is made because there is a difference in behavior; the latter is less aggressive, with a much lower rate of recurrence, in the orthokeratotic Odontogenic Keratocyst , a prominent granular layer is found immediately below a flat noncorrugated surface[4],the basal cell layer is less prominent , with a more flattened or squamous appearance in comparison with the parakeratotic type [4],differences in the clinical behavior of cysts may be associated with apoptosis in the lining epithelium[1,5,6].

The nomenclature and classification of odontogenic tumors are based on those recommended by the World Health Organization (WHO) (1992) and its Concensus Conference, (2003), the latter redesignated the OKC as the (KCOTs) keratocysticodontogenic tumor[1].

The Bcl-2 proto-oncogene is a member of a gene family located in chromosome 18 codes for 26 KD protein that includes cell death suppressors and cell death promoters[5,6],the protein Bcl-2, is a 26 kDa putative membrane associated protein which acts as a cell death suppressor that facilitates cell survival by regulating apoptosis. Investigations on the immune reactivates of

Bcl-2 protein have been demonstrated in tooth germs, ameloblastomas, Odontogenic Keratocycts and dentigerous cysts [5,6,7].

Odontogenic Keratocycts present an aggressive clinical course with a marked trend toward recurrence as compared with dentigerous cysts. Differences in the clinical behavior of cysts may be associated with apoptosis in the lining epithelium [8], therefore we make this study to see the expression of apoptosis-related factor Bcl-2 in Odontogenic Keratocyct by immunohistochemical analysis [8].

Chapter 2

REVIEW OF LITERATURES

2.REVIEW OF LITRATURE

2.1 History of OKC

Odontogenic kerato cyst (OKC), described by Philipsen in 1956[3].
was recently designated by the World Health Organization of the classification of head and neck tumors, published in 2005, has been reclassified the odontogenic kerato cyst as a benign intraosseous neoplasm, recommending the term kerato cystic odontogenic tumor (KCOT), and was defined as "a benign uni- or multicystic, intraosseous tumor of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behaviour[9,10,11]. It may occur in any part of jaws with most lesions occurring in the mandible, most commonly in the posterior body and ascending ramus [10,11]. The OKC is one of the most aggressive odontogenic cysts owing to its relatively high recurrence rate and its tendency to invade adjacent tissue [3].

2.2 Current concepts of odontogenic tumours

Odontogenic tumours are a group of lesions that arise from the tissues derived from the tooth-forming apparatus. They are thus exclusive to the jaws and represent the only situation in pathology where a primary epithelial tumour may be found within bone. Odontogenic tumours are rare and lack of familiarity with these lesions and their variable appearance may lead to difficulties in diagnosis with occasional serious confusion with more sinister lesions [12].

The classification of Odontogenic tumours is based first on behaviour, benign or malignant, and then on the histomorphogenesis of the lesions. As a group they are derived from the epithelial, ectomesenchymal and mesenchymal

tissues that are part of the tooth-forming apparatus. Thus odontogenic tumours are divided into lesions derived from odontogenic epithelium only, tumours derived from odontogenic mesenchyme only and those composed of both odontogenic epithelium and mesenchyme[12]. The appearance of the lesions and thus their classification also depends on the degree of interaction between the epithelial and mesenchymal components; this interaction, often referred to as inductive change, may result in cytodifferentiation that recapitulates the structures of the normal tooth-forming apparatus or enamel organ [12].

In some lesions, especially those composed of both odontogenic epithelium and mesenchyme, formation of the dental hard tissues, enamel and dentine, is commonly seen. The presence of ameloblast-like cells and the formation of dental hard tissues, especially dentine, are useful indicators of the odontogenic origin of epithelial lesions encountered within the jaws [12].

2.3 Epidemiology of OKC

Odontogenic kerato cyst occur over a wide age range, but peak incidence in the second and third decades with a second smaller peak in the fifth decade[1]. The cysts are more common in males than females, and 70-80 per cent occur in the mandible [1]. The most common site accounting for at least 50 per cent of all cases, in the third molar region and ascending ramus of the mandible, in maxilla the majority of cysts occur in the region posterior to the first premolar [1].

OKC are relatively rare. In an analysis of cases received in the Oral & Maxillofacial Pathology in Sheffield- Uk, odontogenic tumours comprised only 1% of all specimens and 5% of jaw lesions. This gives an estimated incidence of less than 0.5 cases per 100,000 per year. The most frequently encountered intra-osseous lesions of the jaw (80%)were the odontogenic cysts [3].

The relative frequency of the different odontogenic tumours varies from country to country [12].The most common true neoplasm is the ameloblastoma, comprising less than 20% of all odontogenic tumours. The next most common, at about 3–4% each, are fibromas, myxomas, adenomatoidodontogenic tumours (AOTs) and ameloblastic fibroma[12].Other tumours, such as calcifying epithelial odontogenic tumour (Pindborg tumour) and ghost cell lesions, may comprise only about 1% of the total and are therefore very rarely seen [12].Another a study of fifty-one cases of OKC conducted by Duangrudee and his colleagues in Bangkok from 1988 to 2003 were studied retrosoectively showed that the odontogenic kerato cysts (OKCs) are epithelial developmental cysts[3]. It occurs mainly in the second and third decades, with a slight male predilection. Furthermore, frequency of OKC has been reported to vary from 3% to 11% of odontogenic cysts [3]. It occurs mainly in the second and third decades, with a slight male predilection [3].

2.4 Clinical features of OKC

Odontogenic Kerato cyst occur over a wide age range, but there is pronounced peak incidence in the second and third decades with a second smaller peak in the fifth decade. The cysts are more common in males than females[1], it occurs most commonly in the third molar region and ascending ramus[1],odontogenic Kerato cyst give rise to remarkably few symptoms, unless they become secondarily inflamed, and this probably accounts for why some do not present until the fifth decade. Contrast to radicular and dentigerous cysts, OKC tend to expand in an anteroposterior direction and can reach large sizes without causing gross bony expansion they are often discovered on routine radiographic examination [1].

The majority of OKC are solitary, multiple cysts are associated with Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder with

a high degree of penetrance and a variable expressivity characterized by several development defects and a predisposition to cancer. (NBCCS) mainly characterized by presence of multiple basal cell carcinoma, odontogenic kerato cysts of the jaw, and volar, acral pits[1,13, 14].

Some studies have even suggested that the odontogenic kerato cyst (OKC) is a benign neoplasm occurs sporadically or in association with nevoid basal cell carcinoma syndrome (NBCCS) or Gorlin–Goltz syndrome this syndrome is associated with a wide developmental anomalies and neoplasms [1,14].

This lesion arises from the dental lamina epithelium and occurs most commonly in the mandible [10,11,13]. OKC has an aggressive clinical behavior with extensive local invasion and a high recurrence rate [10,11,13].

2.5 Radiographicallyof OKC

OKCs present as a well-defined radiolucent lesions with smooth and usually corticated margins. They may present as either a multilocular or unilocular radiolucent lesion,most commonly associated to an unerupted tooth [3].

2.6 Pathogenesis of OKC

Pathogenesis of OKC is generally agreed that the OKC is an abnormality arising from Odontogenic epithelium .most of the available evidence points to two main sources of epithelium from which the cyst is derived:

- The dental lamina or its remenants
- Extensions of basal cells from the overlying oral epithelium [14].

The pathogenesis of the odontogenic cyst is concluded that the dental cyst arises from proliferation of the epithelial rests of Malassez in a focus of inflammation stimulated by pulpal necrosis of the associated tooth. It enlarges by unicentric expansion from the hydrostatic pressure of its contents.

One of the characteristic features of growth of this pathology is tendency to grow along the cancellous channels with very little cortical expansion varios theores of expansion of KCOT have been proposed to explain this, these include intraluminal hyper osmolality [(15)].

2.7 Histopathologic features:

The histological features are characterized by the presence of a thin band like parakeratinized or orthokeratinized stratified squamous epithelium, with a prominent basal layer composed of either columnar or cuboidal cells, and connective tissue wall that is usually free of inflammation [(3)]. The parakeratotic type forms 85 to 95% of all odontogenic kerato cysts; the balance is made up of the orthokeratinzed variant. Histological distinction between the para-and orthokerat-inzed variants is made because there is a difference in behavior; the latter is less aggressive, with a much lower rate of recurrence [(3)].

It's typical histological features include a thin parakeratinized squamous epithelium, approximately 5–8 cells thick, covered by a thin corrugated layer of parakeratin 2–4 the basal layer exhibits a characteristic palisaded pattern with uniform nuclei. The epithelium can show budding of the basal layer into surrounding connective tissue with formation of detached micro cysts, which have been termed daughter cysts [(10)].

The fibrouscyst wall is relatively thin and usually lacks inflammatory cell infiltrate. Malignant transformation into squamous cell carcinoma, though rare, has been reported [(3.10)],While Pieterin 2006 [(16)], proved that Kerato cystic odontogenic tumour (KCOT), formerly known as odontogenickerato cyst but now renamed to emphasize its neoplasic nature, shows a thin connective tissue wall lined by stratified squamous epithelium with a well-defined basal layer of palissading columnar or cuboidal cells and with a corrugated surface layer of parakeratin [(16)] .

The underlying cyst wall may contain tiny daughter cysts and solid epithelial nests,when inflamed, the typical histological features are replaced by a non keratinizing stratified epithelium exhibiting spongiosis and elongated rete pegs supported by connective tissue containing a mixed inflammatory cells infiltrate [16],rarely, KCOT shows development of epithelial dysplasia and squamous cell carcinoma[16].

Occasionally, intraosseous cystic lesions are lined by orthokeratinized epithelium; thus having the appearance of an epidermoid cyst, these orthokeratinizedodontogenic cysts do not form part of the spectrum shown by KCOT also, KCOT tends to recur after enucleation[16]. If associated with the nevoid basal cell carcinoma syndrome, the chance of recurrence is even higher.

Furthermore, markers known to be rapidly induced in response to growth factors, tumor promoters, cytokines, bacterial endotoxins, oncogenes, hormones and shear stress, may also shed new light on the biological mechanisms involved in the development of these benign but sometimes aggressive neoplasm of the jaws [10].

2.8 Differential diagnosis of OKC

When cysts are associated with teeth, several entities might be considered, such as dentigerous cyst, ameloblastoma, odontogenicmyxomas, adenomatoi dodontogenic tumour, and ameloblastic fibroma. Lucent non odontogenic tumour, such as central giant cell granuloma, traumatic bone cyst, and aneurismal bone cyst, might be included in a differential diagnosis of this entity in young patients [4].

2.9 Treatment and prognosis OKC

There is a wide variety of surgical approaches depending on the size and extent of the lesions, including decompression, curettage, marsupial-lization, enucleation or resection, with more meticulous surgical approaches correlating to a better prognosis,in the literature there is a controversy regarding the treatment of this lesion: some surgeons advocate conservative therapies, whereas others are in favor of an aggressive treatment [10,11], therapeutic approaches vary in different studies from marsupialization and enucleation, which may be combined with adjuvant therapy such as cryotherapy or Carnoy's solution, to marginal or radical resection[3]. The recurrent rate varies from approx-imately 20% to 62% [3].

Moreover, all types of treatment, except marginal resection, gave rise to recurrence. The recurrent lesions occurred more frequently in parak-eratinized OKCs, symphysis-body region, and patients who had lesions associated with the remaining teeth and were treated by enucleation and enucleation with curettage. Recurrence rate was common . The interval between primary surgery and presentation of recurrence ranged from 2 to 10 years; 71.4% occurred within 5 years. Long-term follow-up at regular intervals after surgery is recommended [3],in Italy a study conducted by Salvatore et al in 2009[11],have suppo-rted the conservative management includes enucleation curettage marsup-ialzation, and decompression [11].

Aggressive therapies consist of enucleation with chemical curettage or resection with or without loss of jaw continuity, endoscopy is now used by otorhino laryngologists and oral and maxillofacial surgeons for several conditions such as craniofacial surgery, cosmetic surgery, temporo-mandibular joint surgery, sinus surgery, laryngeal surgery, salivary gland surgery, and trauma surgery[11]. Endoscopic techniques allow for a greater

visualization of the operative field by magnifying the view through the aperture of telescopic lenses and transferring the image to a television monitor[11]. As minimally invasive surgery, the benefits of endoscopy include, in addition to a direct visualization of a magnified and illuminated operative field for the surgeon, performing operations with small access and decreased morbidity [11].

Most recurrences become clinically evident within 5 years of treatment. A side from recurrence potential, ameloblastic transformation is a rare complication. Patients with multiple kerato cysts have a significantly higher rate of recurrence than do those with single kerato cyst (30%and 10% respectively) [4].

2.10 Genetic mechanisms in the development and progression of kerato cystic odontogenic tumor (KCOT).

Morphogenesis and cyto diffrentiation of the teeth are under genetic control of regulators such as Sonic Hedgehog (SHH), bone morphogenetic protein (BMP), and tumor-suppressor genes acting as regulators of cell growth, possible molecular mechanisms have been investigated for elucidation of many biological aspects of OKCs[10],loss of heterozygosity in the region 9q22.3and mutations in the tumor suppressor gene Patched (PTCH) were identified as genetic alterations in sporadic OKCs and associated with NBCCS. However, other factors related to regulation of genes may affect the development of OKCs [9].

In activation of these genes by mutations and/or loss of heterozy-gosity(LOH) results in tumor development. Expression of Hedgehog signaling molecules – SHH, PTCH, smoothened (SMO), and GLI1 –has been detected in several odontogenic tumors, suggesting that SHH signaling pathway plays a role in epithelial–mesenchymal interactions and cell proliferation during the growth of odontogenic tumors as well as during tooth development [10].

The PTCH encodes a transmembrane protein implicated in the Sonic Hedgehog (SHH) signal transduction pathway, controlling cell fates, patterning, and growth in numerous tissues, including teeth [10],PTCH is thought to combine with Smoothened (SMO) to form a trans-membrane receptor complex which acts as the receptor for SHH ligands. When SHH signal binds to PTCH, which normally represses SMO, this inhibition is released, allowing SMO to activate the Gli-family zinc-finger transcription factors (GLI1), resulting in up regulation of the transcription of cellular proli-feration genes. Alterations, either inherited or sporadic, in the SHH signaling pathway genes might cause a number of developmental defects [10].

2.10.1 The Bcl-2 family

Consists of a number of evolutionarily-conserved proteins that share Bcl-2homology (BH) domains, the Bcl-2 family is most notable for their regulation of apoptosis, a form of programmed cell death, at the mitochon-drion,the bcl-2 family proteins consists of members that either promote or inhibit apoptosis, and control apoptosis by governing mitochondrial outer membrane permeabilization (MOMP). There are a total of 25 genes in the Bcl-2 family known to date [17].

Bcl-2 family proteins have a general structure that consists of a hydrophobicα-helix surrounded by amphipathic α-helices. Some members of the family have transmembrane domains at their c-terminus which primarily function to localize them to the mitochondrion. Bcl-x(L) is 233 amino acyl residues (aas) long and exhibits a single very hydrophobic putative transmembrane α-helical segment, when in the membrane. Homologues of Bcl-x include the Bax (rat; 192 aas) and Bak (mouse; 208 aas) proteins, which also influence apoptosis[17, 18].

The high resolution structure of the monomeric soluble form of human Bcl-x(L) has been determined by x-ray crystallography,the structure consists of two central primarily hydrophobic α-helices surrounded by amphipathic helices[18]. The arrangement of the α-helices in Bcl-X(L). The colicins similarly form pores in lipid bilayers[18]. Structural homology therefore suggests that Bcl-2 family members that contain the BH1 and BH2 domains (Bcl-X(L) Bcl-2 and Bax) function similarly [18].

The Bcl-2 inhibits apoptosis to facilitate cellular proliferationin the basal and supra basal layers, where as apoptosis maintains the homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs[19],thus, Bcl-2 family is recognized as a cell division regulator, which can inhibit apoptosis and produce extended cell survival [19].

Relevant classes of apoptosis-regulatory gene products. Bcl-2 and bax are widely regarded as the most important apoptotic regulators, and their relative levels determine the fate of cells. Bcl-2 protein expression in the mitochondrial outer membrane inhibits cytochrome translocation into the cytosol, which is a critical step in the apoptotic process [19], OKC were characterized by higher expression of Bcl-2 in basal cell epithelium. Unicystic ameloblastoma differed from OKC in a wide spect-rum of apoptosis and/or cell cycle-related protein expressions, higher proliferation in the basal cell layer, and vice versa, lower proliferation in the supra basal cell layer. Unicystic ameloblastoma Bcl-2 protein is chara-cterized by its ability to inhibit apoptosis [19].

Considering the fact that bcl-2 over expression may lead to increased

survival of epithelial cells[20]. A study may demonstrate a possible relationship between the aggressive nature of OKC and the intrinsic growth potential of its lining epithelium. Furthermore a basal/supra basal distribution of bcl-2 positive cells was seen in some odontogenic kerato cysts which may have a significant impact on the behavior of this cyst[20].

2.10.2 Function of apoptosis

Active cell suicide (apoptosis) is induced by events such as growth factor withdrawal and toxins. It is controlled by regulators, which have either an inhibitory effect on programmed cell death (anti-apoptotic) or block the protective effect of inhibitors (pro-apoptotic) [19].

Apoptosis is a cell suicide mechanism that occurs during embryonic development, in path physiological conditions. The apoptotic process is controlled by several gens, including inducers (p53, Bcl-xs, Bax, Bak) and repressors Bcl-2 family (Bcl-2,Bcl-xs.Bcl-x1,Mcl-1, Bax, Bak). The balance between these stimuli regulates the cell cycle and programmed cell death (apoptosis) [21].

Many viruses have found a way of countering defensive apoptosis by encoding their own anti-apoptosis genes preventing their target-cells from dying too soon. Bcl-x is a dominant regulator of programmed cell death in mammalian cells. The **long form** (Bcl-x(L), displays cell death repressor activity, but the **short isoform** (Bcl-x(S)) and the β-isoform (Bcl-xβ) promote cell death. Bcl-x(L), Bcl-x(S) and Bcl-xβ are three iso forms derived by alternative RNA splicing[21].

There are a number of theories concerning how the Bcl-2 gene family exert their pro- or anti-apoptotic effect. An important one states that this is

achieved by activation or inactivation of an inner mitochondrial permeability transition pore, which is involved in the regulation of matrix Ca^{2+}, pH, and voltage[20]. It is also thought that some Bcl-2family proteins can induce (pro-apoptotic members) or inhibit (anti-apoptotic members) the release of cytochrome c into the cytosol which, once there, activates caspase-9 and caspase-3, leading to apoptosis[22].

Although Zamzami etal in 1998[22], suggest that the release of cytochrome c is indirectly mediated by the PT pore on the inner mitochondrial membrane, strong evidence suggest an earlier implication of the MAC pore on the outer membrane, another theory suggests that Rho proteins play a role in Bcl-2**,** Mcl-1 and Bid activation [22]. Rho inhibition reduces the expression of anti-apoptotic Bcl-2 and Mcl-1 proteins and increases protein levels of pro-apoptotic Bid but had no effect on Bax or FLIP levels. Rho inhibition induces caspase-9 and caspase-3-dependent apoptosis of cultured human endothelial cells [22].

2.10.3 Apoptotic mechanisms

Apoptosis the TUNEL method which is based on the addition of labeled UTP to the 3 ends of fragmented DNA by TdT[23]. Previous reports comparing apoptosis-related factors in sporadic KCOTs and KCOTs associated with nevoid basal cell carcinoma have been published1and apoptotic cells have been found in the superficial cells of the lining epithelia of KCOTs through the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method[23].

Among all proto-oncogenes, bcl-2, located at chromosome 18q21, characteristically able to stop programmed cell death (apoptosis) without promoting cell proliferation. Its gene product, the bcl-2 protein,

acts as a cell death suppressor that facilitates cell survival by regulating apoptosis.Investigations on the immunoreactivities of Bcl-2 protein have been demonstrated in tooth germs, ameloblastomas, KCOTs and dentigerous cysts [24].

Recent studies (2010) reported that Bcl-2 positive cells are predominantly located basally, thus supporting the concept that apoptosis does not occur in the basal cells of the lining epithelium. positive cells have been detected exclusively in the surface layer of KCOTs, indicating marked levels of apoptosis[10] Thus, Bcl-2 inhibits apoptosis to facilitate cellular proliferation in the basal and supra basal layers, whereas apoptosis maintains the homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs[10].Considering that there is a regulated balance between cell proliferation, cell differentiation and cell death in this type of lesion, this may explain why KCOTs, though portraying a neoplastic behavior, with an increase potential to proliferate, do not tend to form tumor masses [10].

Genetic alterations are considered important events in benign and malignant tumors of head and neck. The genes can be modified through this mechanism, without having their DNA sequences changed [9], on the other hand, there are no published data about the genetics of the OKC. These facts together prompted them to investigate the presence of methylation in P16, P21, P27, P53 and RB1 genes in OKC tumors [9].

From dental literature recorded that ameloblastomas and odontogenic cysts like kerato cystic odontogenic tumor and dentigerous cysts are often derived from the epithelial remnants and follicles and this suggests that

early intervention of removing the impacted teeth and associated follicles can reduce pathologies to a certain extent [25,26].

In addition, epidermal growth factor receptor (EGFR) has been shown an importance in the genesis and behavior of some types of tumor like solid multi locular ameloblastoma. Ki-67 is a molecule that can be easily detected in proliferating cells in order to gain an understanding of the rate at which the cells within a tumor are growing [27] typically 8-10 cells layers thick-with relatively uniform thickness, lacks rete ridges and palisaded basal cell layer. Whereas the key feature is Parakeratosis (keratinized cells with nuclei)[28].

When compared with other study the histological appearances are characteristic, the cyst is lined by a thin, regular stratified epithelium From literature the features of OKCs are Stratified Squamous epithelium (resembling squamous epithelium) with: "**Ribbon**-like appearance" ,finely corrugated parakeratinized surface. The epithelial lining is folded and may appear to extend in long finger-like processes[28]. Many cysts show small islands of odontogenic epithelium in the wall and there may be budding of the basal. Results from Japan in 2004 [29], suggested that odontogenic kerato cyst (OKC) had high cellular proliferation and was relatively low differentiation as compared with orthokeratinized odontogenic kerato cyst (OOC) and epidermal cyst (EDC) [29].

In addition, there was participation of apoptosis suppressor in OKC. The character of orthokeratinized odontogenic kerato cyst occupied an intermediate position between odontogenic kerato cyst and epidermal cyst Therefore, it was possible to consider orthokeratinized odontogenic kerato cyst as a distinct entity from OKC, epidermal cyst had the lowest cellular activity in the three cysts and it was comparatively maturated lesion [29].

Bcl-2 has derived its name from B-cell Lymphoma-2; a second member of range of proteins initially described on chromosomes **14 and 18** in follicular lymphomas[29]. (It is a proto-Oncogene that produces a protein found in endoplasmic reticulum, nuclear envelope and mitochondrial membrane. It is considered as anti-apoptotic protein which prolongs survival of cells by blocking apoptosis and promoting development of tumor[29].

Chapter 3

AIMS OF THE STUDY

3. Aims of Study

1- To assess the clinic histopathological features of Odontogenic Kerato cyst cases.

2- To study the immune -expression of Bcl-2 in Odontogenic Kerato cyst.

Chapter 4

MATERIALS AND METHODS

4. Materials and Methods

4.1 Study design, sample size study and period:

A descriptive case-series study.of cases diagnosed of OdontogenicKeratocyst (OKC) including ortho and para Odontogenickeratocyst by histopathology and confirming to KCOTs by the new classification (WHO, 2005) were selected for this study,the data were collected from the archives of oral pathology department, faculty of dentistry, university of Benghazi –Libya in the Period from January 1997 to December 2014.The clinical data such as age, sex, residence, nationality and site of the lesion were noted from the patients records, a convenient sample of twenty patients (n = 20) diagnosed as OKCs, the period of this study was nine months.

4.2 Study setting:

The 20 cases were retrieved from paraffin-wax blocks in archives of oral pathology in the faculty of dentistry at Benghazi University then stained with Haematoxylin and Eosin (H and E) stain , four microns thick sections where cut and mounted on poly L-lysine(coated) glass slides,after that the samples stained by immune marker (Bcl2) using avedin-Bioten-complex technique (**ABC**) to study immune reaction,the data were collected and reviewed from patients charts from archive of oral pathology department, Clinical and epidemiological data such as age , site, gender, and nationality were collected.

4.3 Materials:

1- Antibodies : anti bcl-2 antibody.
(Thermo Fisher scientific Anatomical pathology Rev 120611D)

2- Coated glass slides.

3- Kit for Avedin-Bioten-Complex (**ABC**).
(Thermo Fisher scientific Anatomical pathology Rev 120611D)

4.4 Methods:

Four microns thick sections will be cut and stained with Hematoxylin and Eosin (H & E) according to (Cullin and etal 1985)[30],sections where examined to confirm the diagnosis for Odontogenic Keratocyst and to study the histopathological features as showed in the diagnostic chart in appendix.

4.5 Immunohistochemical Staining:

Formalin-fixed, paraffin-embedded tissue blocks were sliced at 4 microns thickness and mounted on coated glass-slides.

1. Sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide for antigen retrival.
2. They were heated in an autoclave(121c,2atm) in 0.01M citrate buffer (PH 6.0) for 10 minutes.
3. After treatment with normal serum; the section were incubated with primary antibodies at 4c overnight.
4. The applied antibodies were anti-bcl-2 monoclonal antibody.
5. The standard labeled streptavidin-biotin-peroxidase complex method was preformed to bind the primary antibody by a histofine SAB-PO kit.

6. Reaction were visualized by immersing the section in 0.03% diamino-benzidine (DAB) solution containing 2mM hydrogen peroxide for 3 to 5 min.
7. The immune staining reaction of bcl-2 were evaluated according to intensity and patterns of distribution.
8. Intensity of staining was graded as weak, moderate and intense reactions .

The section were then counterstained with mayer's hematoxyline and eosin and examined by light microscope, all the data were collected and analyzed using SPSS version 17 in tables and figures.

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4.6 Statistical methods:

Statistical analysis on this descriptive study results was per-formed by the application of the statistical package social science soft ware version 17 (SPSS Inc, Chicago, Il, USA). Data collected and then analyzed and expressed as frequency distributions and then computed in percentages in tables and figures. Simple statistical parameters such as mean, standard deviation, minimum and maximum were done and finally T test is done to confirm the p value.

Chapter 5

RESULTS

5.Results

The total number of odontogenic Kerato cysts (OKC) were twenty cases, males more the females,13 cases were males and 7 cases were females. With M: F ratio was 1.9 : 1 (Figure1),the age of the cases ranged from 18 to 65 years with mean age 33.25 . most of cases were in the second and third decades (Figure 2, table 1),the location of the Odontogenic kerato cyst mainly in the mandible with 15 cases (75%). while 3 cases(15%) in maxilla, Finally only 1case (5%) was in the palate and 1 case (5%) was in the cheek (Figure 3),all of cases were Libyan nationality and most of cases residency from Benghazi, except one case from Tobruk (Figure 4).

The histopathological features of the cyst lining of the cases :-

Surface epithelium was thin in 16 of the cases(80%), while it was thick in 4 cases (20%), (figure 10) ,the basal layer was palisaded in 11 cases(55%) , while it was not palisaded in 9 (45%) cases (figure 8),the surface layer was parakeratinized in 15 cases (75%) (figure 8) and it was orthokeratinized in 5 of the case (25%) (figure 10),mitotic figure was basal in 16 (80%) cases and it was suprabasal in 4 cases(20%) (table 2) (figure 15).

In histopathological features of cyst wall examination there were few inflammatory cells were present in 14 cases (70%) (figure 5), and satellite cells were present in 8 cases(40%) (figure 12) and epithelial residues were present in 8 cases (40%) (table 3).(figure 6),histopathological feature of cyst cavity : the Keratin squamous cells present in 12 cases (60%) (table 3) (figure 11).

Immunohistochemical reactions :-

Regarding to odontogenic Kerato cysts (OKC) immune response to BCl2 factor, in our study, there were 15 (75%) of the cases revealed positive immune reaction to Bcl2 and nearly 5 (25%) cases were negative immune reaction (table 4) (figure 25) ,the positive reactions were graded to week, moderate and intense reaction, there were intense reaction of Bcl-2 in 7 cases(46.7%) (figure 16,18,26,27,32,34), while 5 (33.3%) of the cases of OKC cases were moderate(figure 29,35) and 3 (20%) of the cases were weak immune reaction to BCl-2 (table 5) (figure 20,21,22,23).

BCl2 positive expression in odontogenic Kerato cysts (OKC) according to the region :-

Positive expression of BCl2 was present in both basal and supra basal layers in 12 cases (80%) (figure 19,26,27,28,29,34), while it was positive reaction to BCl2 in the basal layer only in 3 cases (20%) (table 6) (figure 16,17,20,21),all the positive cases were diffuse reaction.

There were nuclear positive expression in 11 cases (73.33%) (table 7) (figure 21, 30,33,35,36), combined nuclear and cytoplasmic present in 4 cases (26.67%)of OKC (table 7) (figure 16,18,26,27,31,34).

There is a relationship between presence of inflammatory cells and positive immune reaction to BCL2 there are 11 cases of OKC are positive reaction with inflammation ,which represent (73.3%) from positive cases , it is highly significance relationship with p value =.000 (Table 8),finally there is significant relationship between Parakeratinized Type of OKC and positive reaction to BCL2 There are 12 cases 60% with p value =0.002 (Table9) otherwise not significant relationship between Ortho Keratinized OKC and Positive reaction to BCL2 there are 3 cases 15% with p value =0.389 (Table 9).

(Table 1) : Age distribution of twenty cases of OKCs in Benghazi.

Age	Minimum	Maximum	Mean	Standared. Deviation
N=20	18 years	65 years	33.25	13.676

(Table 2) : Histopathological features of cyst lining of twenty cases of OKCs.

Histopathology of cyst lining		Number Of cases	Percent
Stratified squamous epithelium	Thick	4	20%
	Thin	16	80%
Basal layer	Palisaded	11	55%
	Not Palisaded	9	45%
Surface layer	Parakeratinized	15	75%
	Orthokeratinized	5	25%
Mitotic figure	Basal	16	80%
	Supra basal	4	20%

(Table 3) :Histopathological features of odontogenic keratocysts (OKCs).

Histopathology			Number Of cases	Percent
Cyst wall	Few inflammatory cells	Present	15	75%
		Absent	5	25%
	Satellite cyst	Present	8	40%
		Absent	12	60%
	Epithelial residues	Present	8	40%
		Absent	12	60%
Cyst cavity	Kerattin squames	Present	12	60%
		Absent	8	40%

Table (4) :Study the immune -expression of Bcl-2 in odontogenic keratocyst cases according to presence of reactions.

Reactions	Number Of cases	Percent %
Positive reaction	15	75%
Negative reaction	5	25%
Total of cases	**20**	**100 %**

(Table 5) : immune -expression of Bcl-2 in Odontoginic Keratocysts according to grades of reactions.

Reactions	Number Of cases		Percent
Positive reaction	Weak	3	**20 %**
	Moderate	5	**33.3 %**
	Intense	7	**46.7 %**
	Total positive	15	**75 %**
Negative reaction	5		**25%**
Total of cases	**20**		**100 %**

Table (6) : positive expression of OKC to Bcl-2 according to region.

Bcl-2 expression according to region.	Number Of cases	Percentage
Basal	3	20 %
Both basal & suprabasal	12	80%
Total	**15**	**100 %**

Table (7) : Immunohistopathplogical features of Bcl-2 in OKCs cases according to involvement.

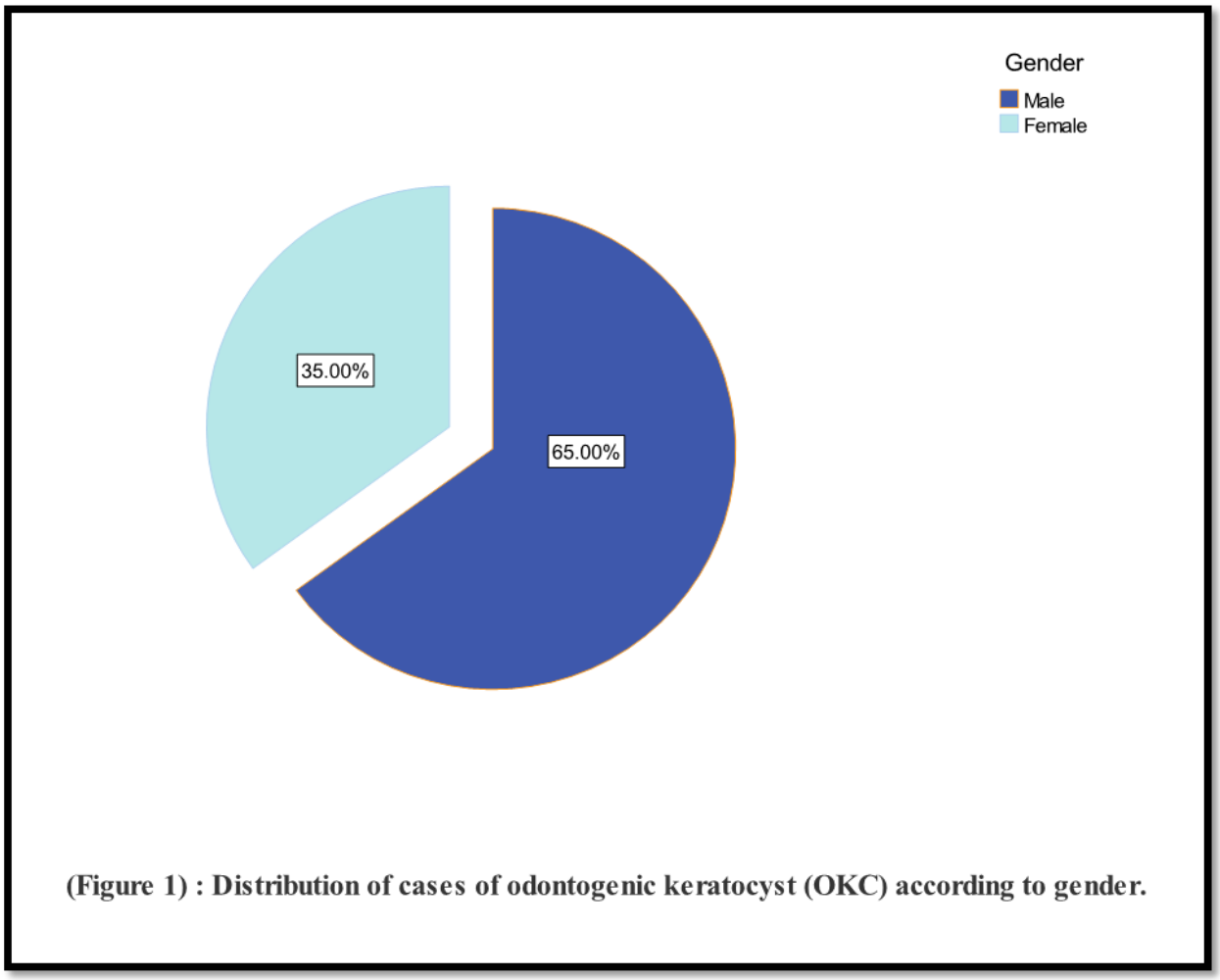
Bcl-2	Number Of cases	Percentage
Nuclear	11	**73.33 %**
Combined (Nuclear, Cytoplasmic)	4	**26.67%**
Total	**15**	**100 %**

Table (8) :Corelation between Presence of inflammatory cells in cyst wall and Immune reactions

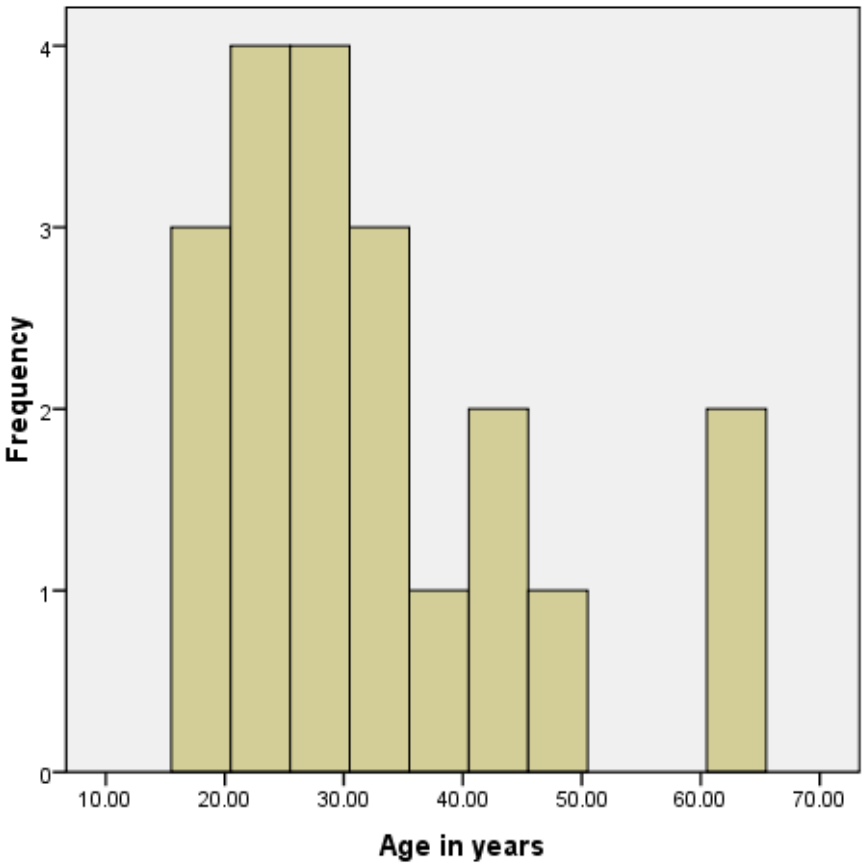
Presence of inflammatory cells in cyst wall	Immune reactions		P-value
	Positive Immune reaction	Negative Immune reaction	
Yes	11	3	.000 (highly Significant)
No	4	2	
Total (%)	15 (75%)	5 (25%)	

Table (9) : Corelation between Parakeratinized and Orthokeratinized (OKC) With Positive Immune reaction

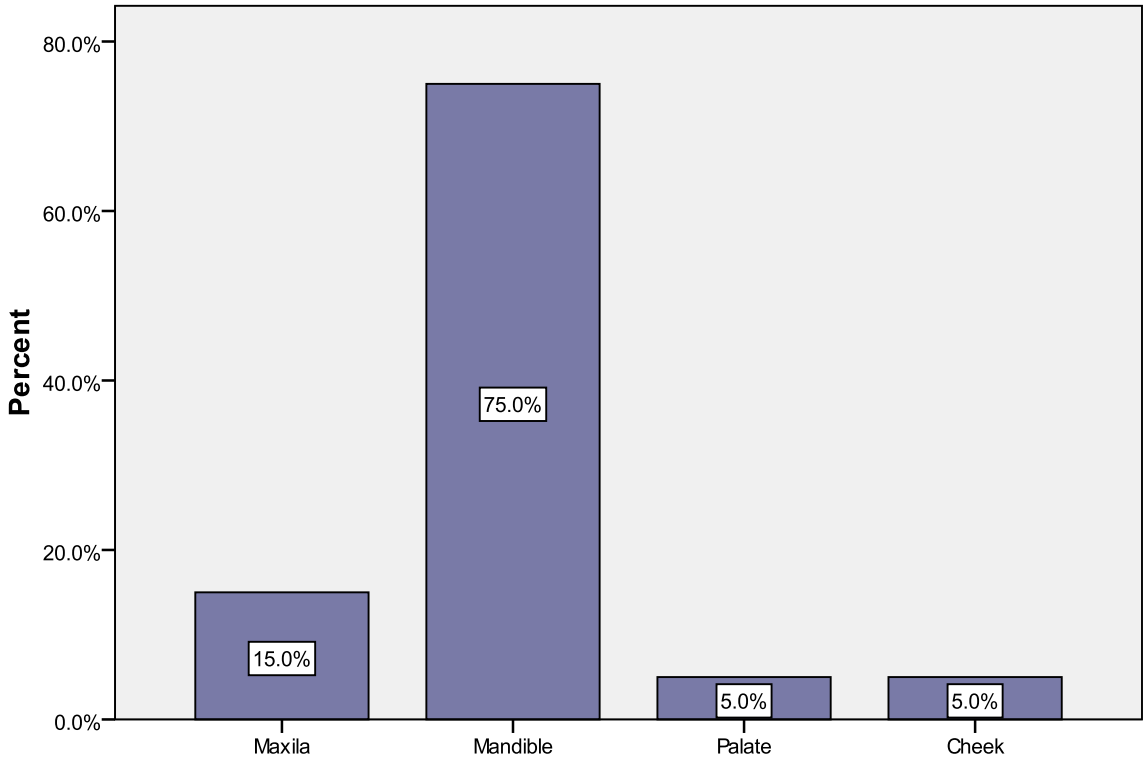
Characteristics Surface layer of Cyst lining	Positive Immune reaction	Percent %	P-value
*Parakeratinized	12	60%	0.002 (Significant)
*Orthokeratinized	3	15%	0.389 (Not significant)



(Figure 1) : Distribution of cases of odontogenic keratocyst (OKC) according to gender.



(Figure 2) : Distribution of patients of OKC according to age.



(Figure 3) : Distribution of odontogenic keratocysts patients according to site of the cysts.

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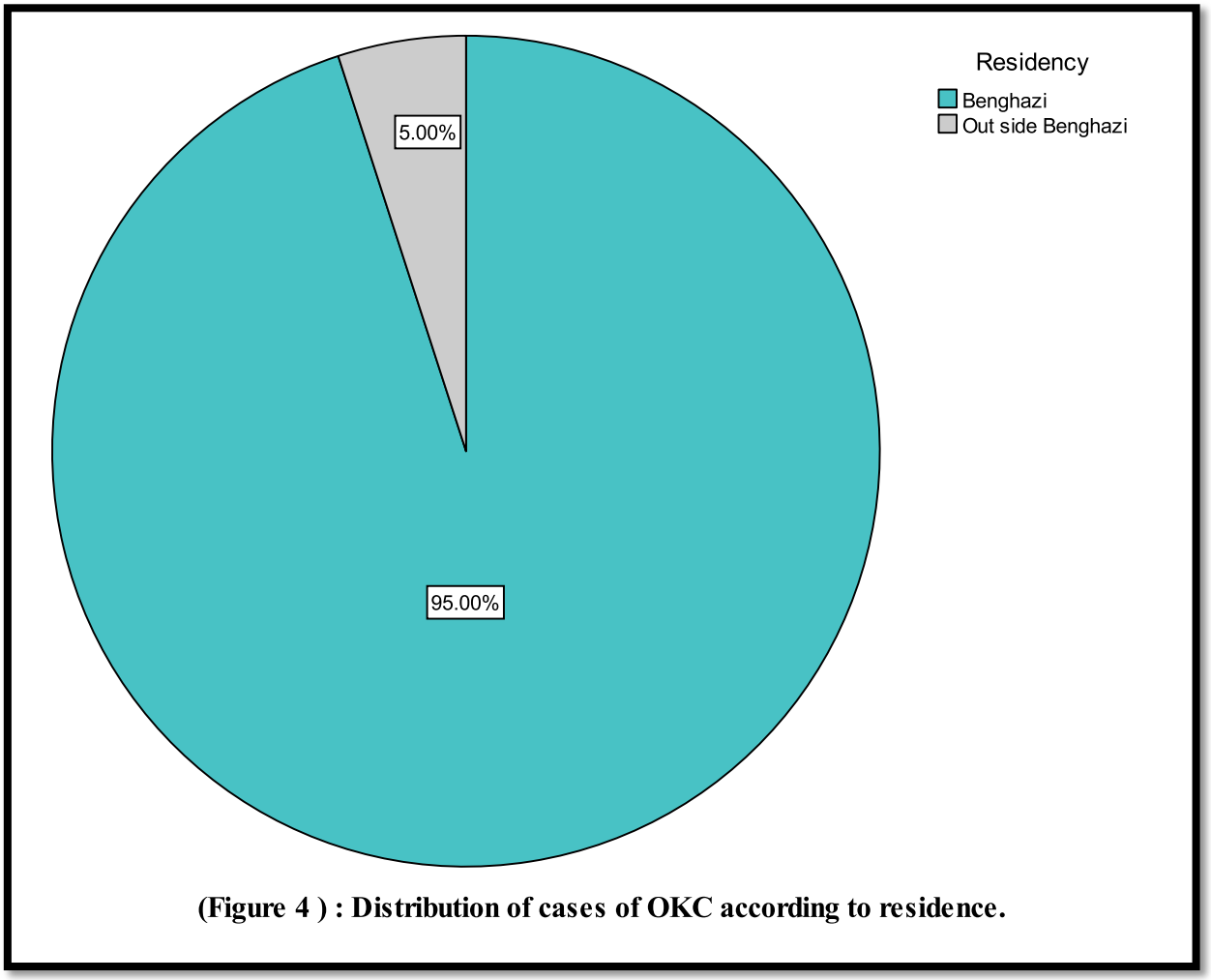
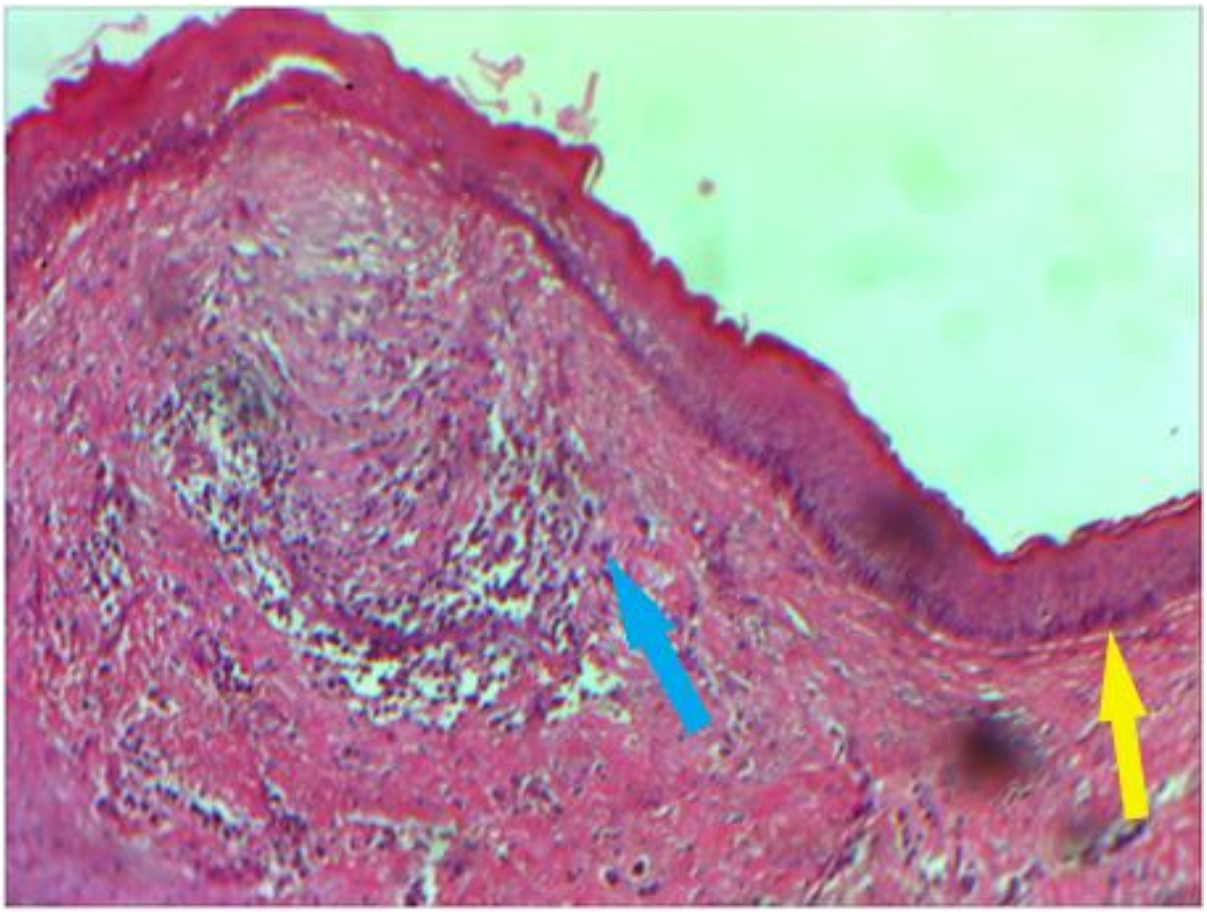
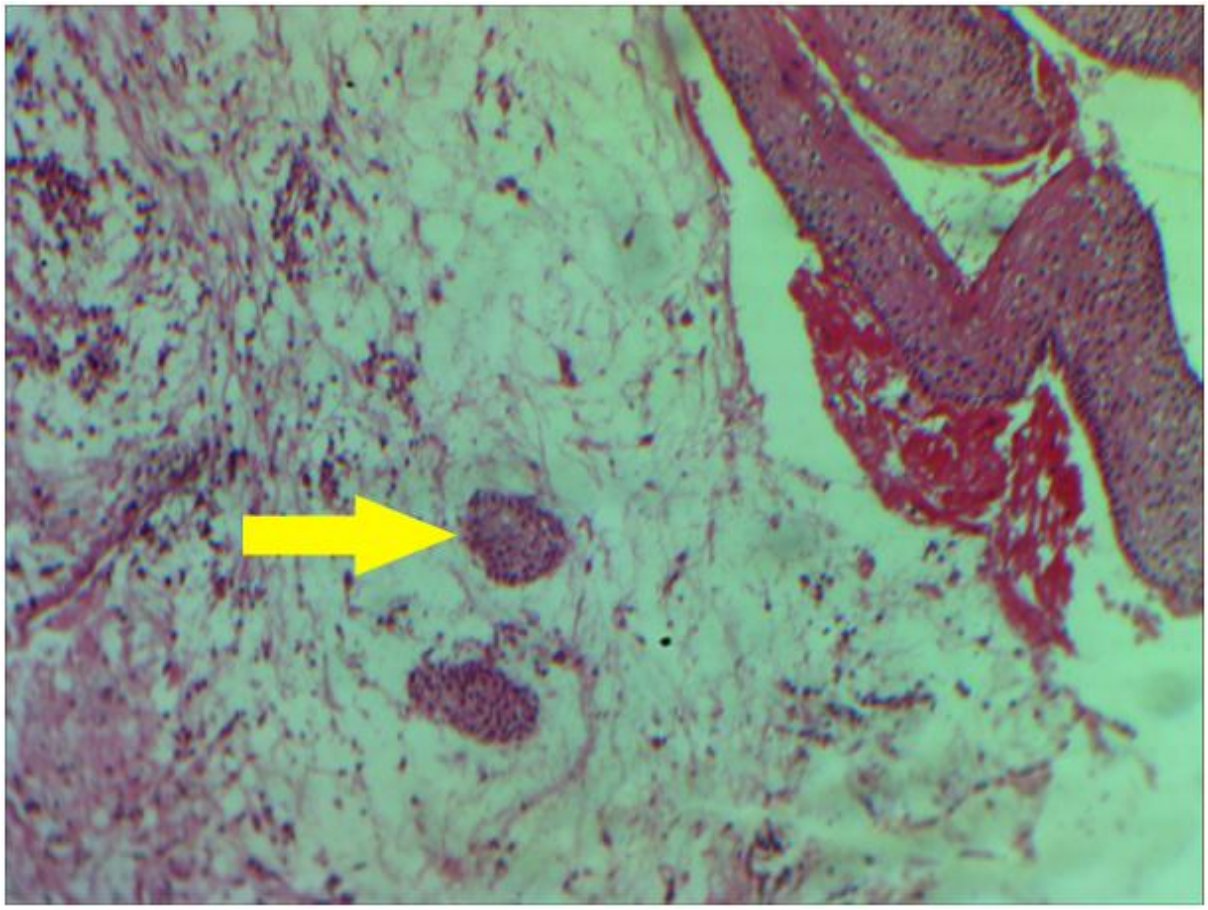


(Figure 4) : Distribution of cases of OKC according to residence.

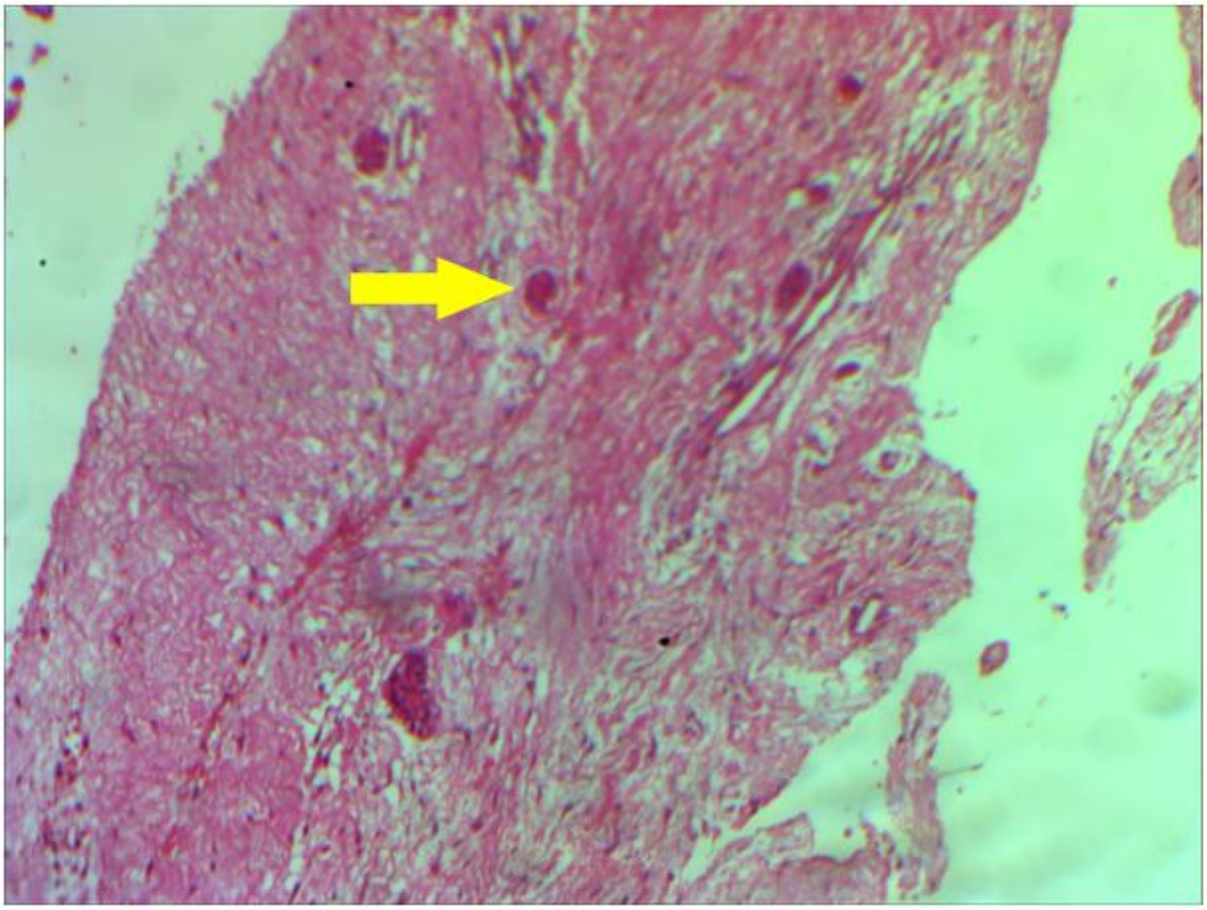
Histopathological Examination of OdontogenicKerato Cyst by Hematoxylin and Eosin (H and E)



Figure(5):Photomicrograph of a histopathological section of odontogenic keratocyst showing palisaded basal cells (Yellow arrow),corrugated parakertnized superficial layer and connective tissue with chronic inflammatory cells (Blue arrow). (Hematoxylin and Eosin ×100).



Fig(6):photomicrograph of histopathological section of OKC shows the cyst wall consists of Epithelial residues(Yellow arrow) (H&E x 100).



Fig(7):photomicrograph of histopathological section of OKC shows the cyst wall consists of epithelial residues (H&E x 100)

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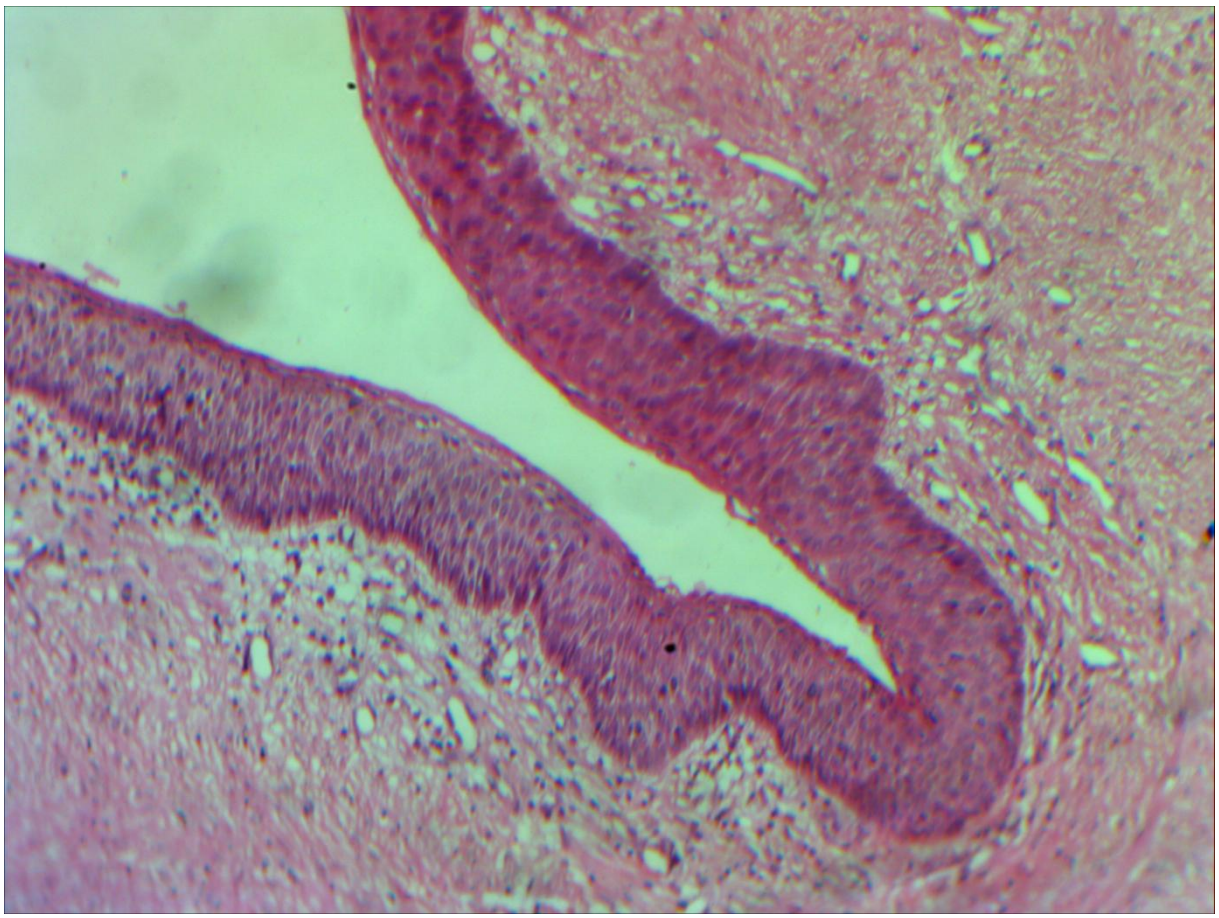
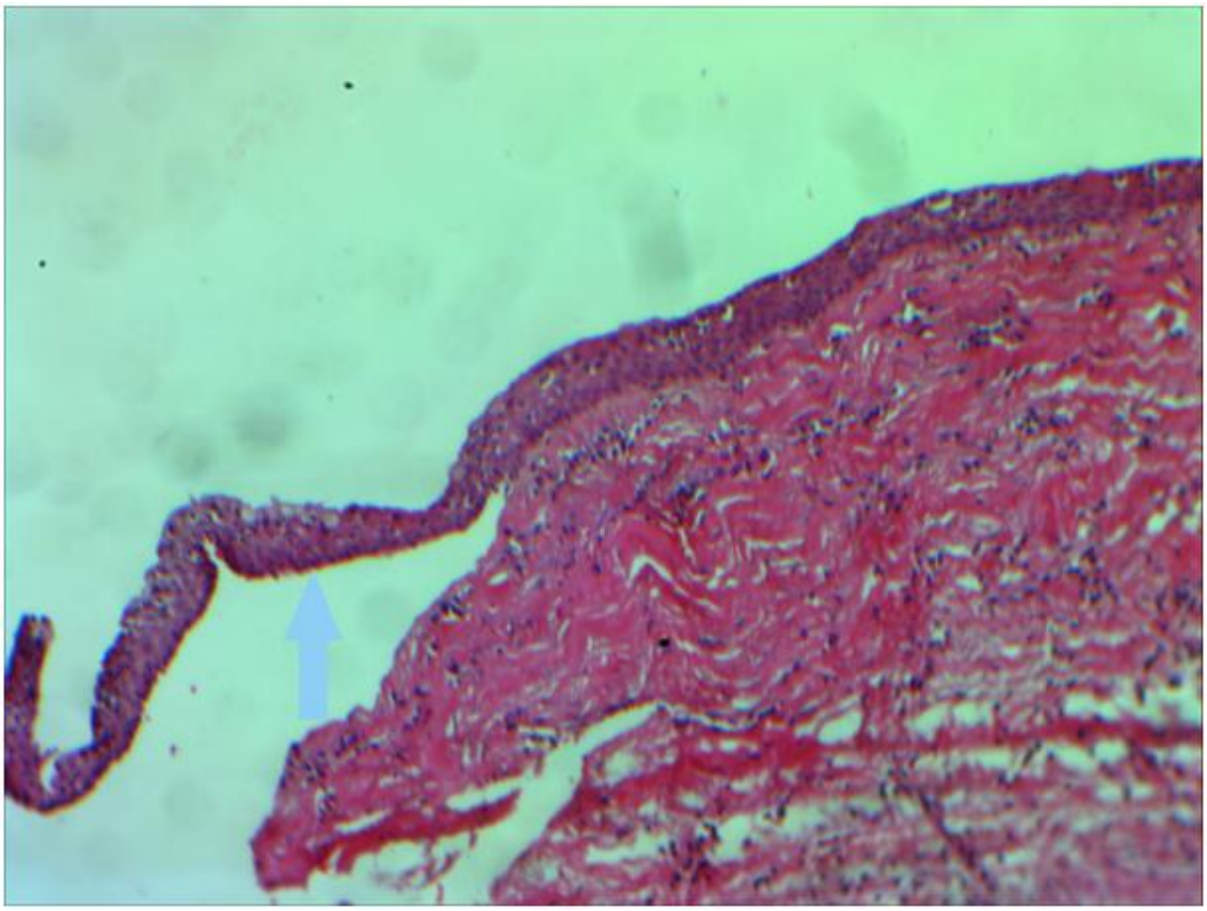
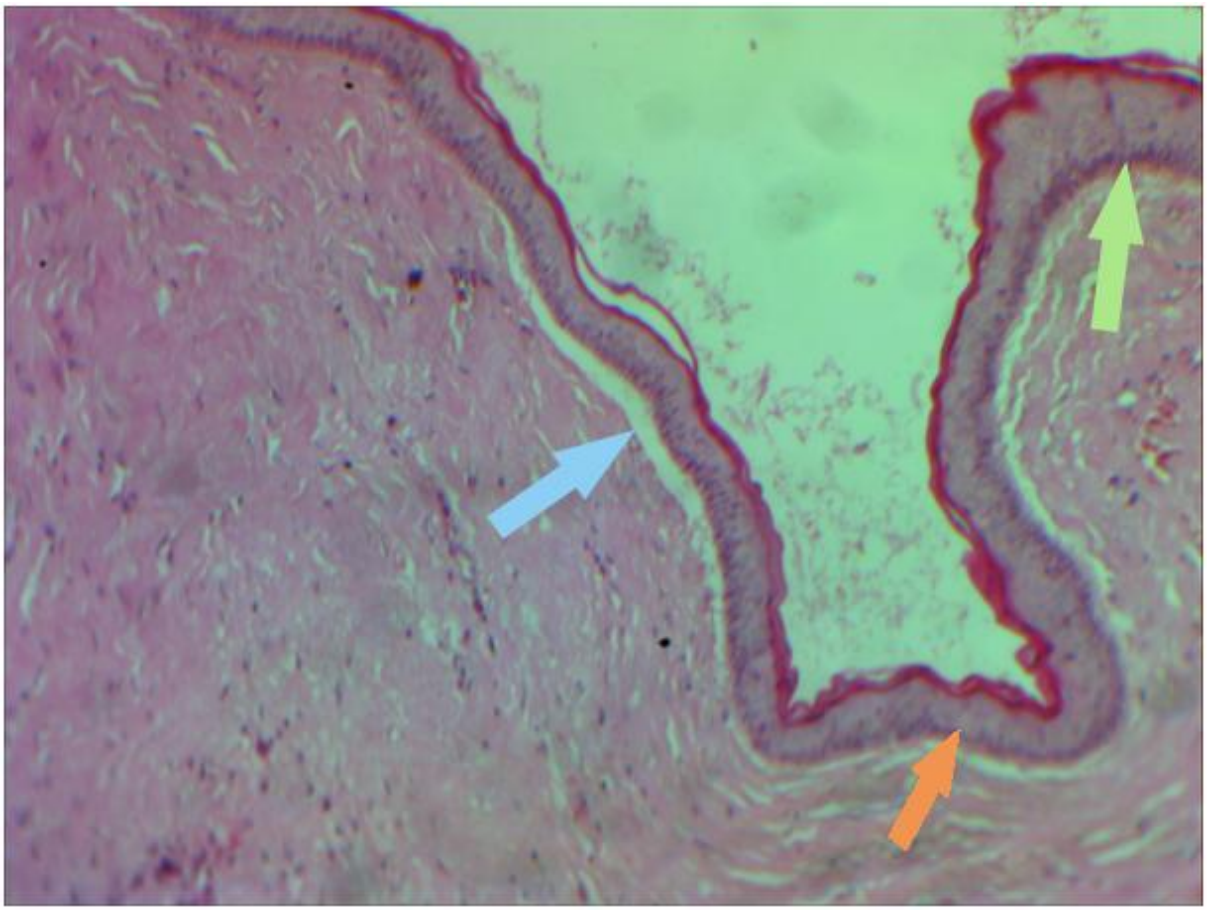


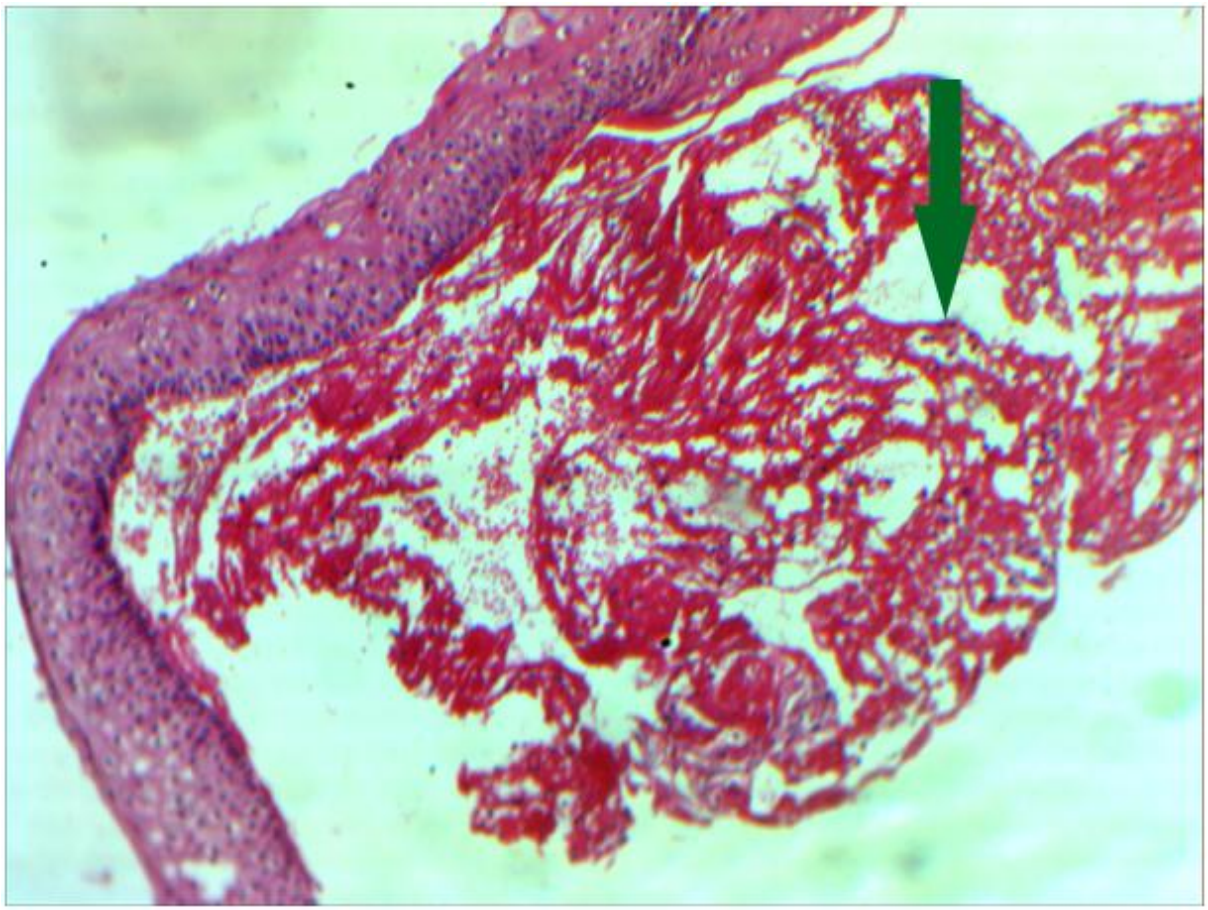
Fig(8):photomicrograph of histopathological section of OKC shows regular parakeratinized epithelium , palaiseded cells , not palsaded cells , (H&E x 100).



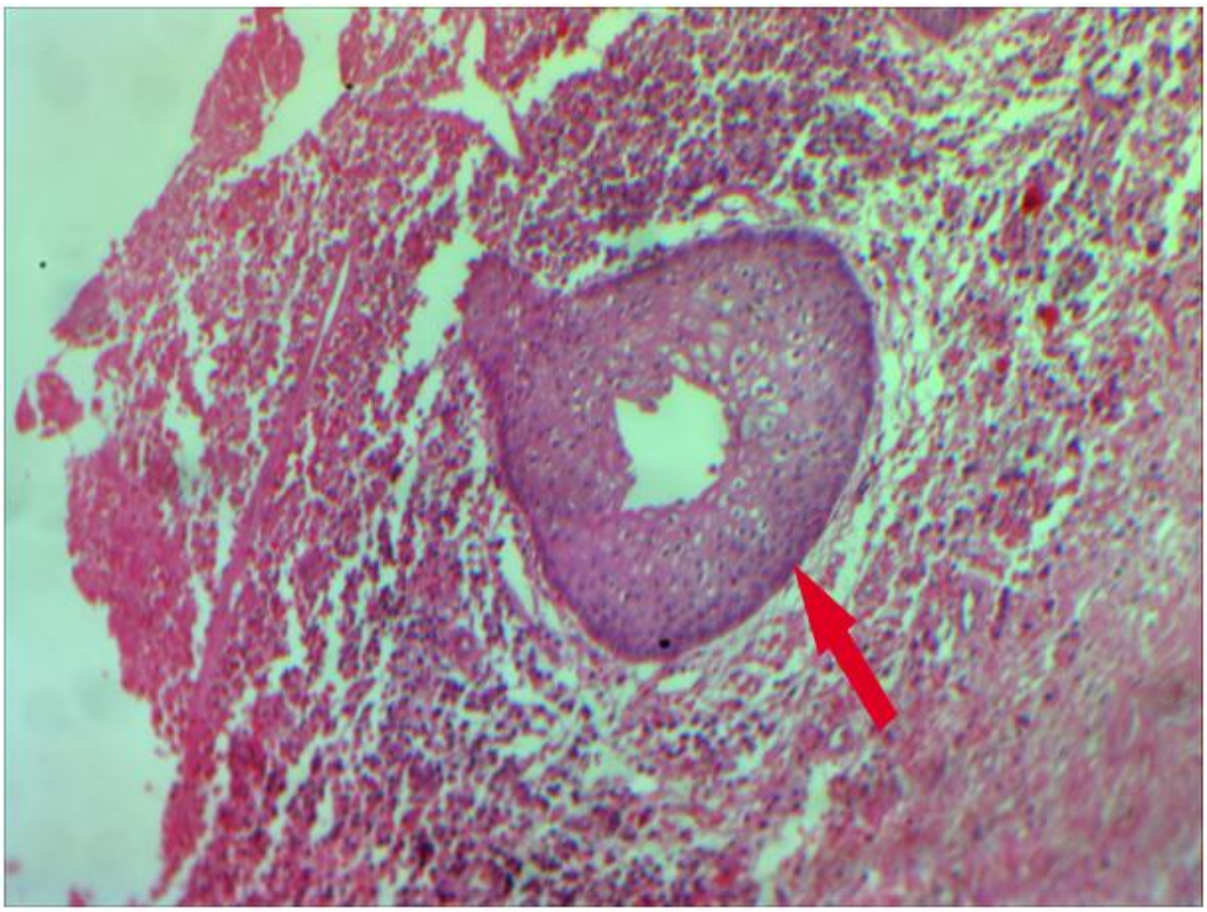
Fig(9): Photomicrograph of histopathological section of OKC shows detached epithelium (Blue arrow) (H&E x 100).



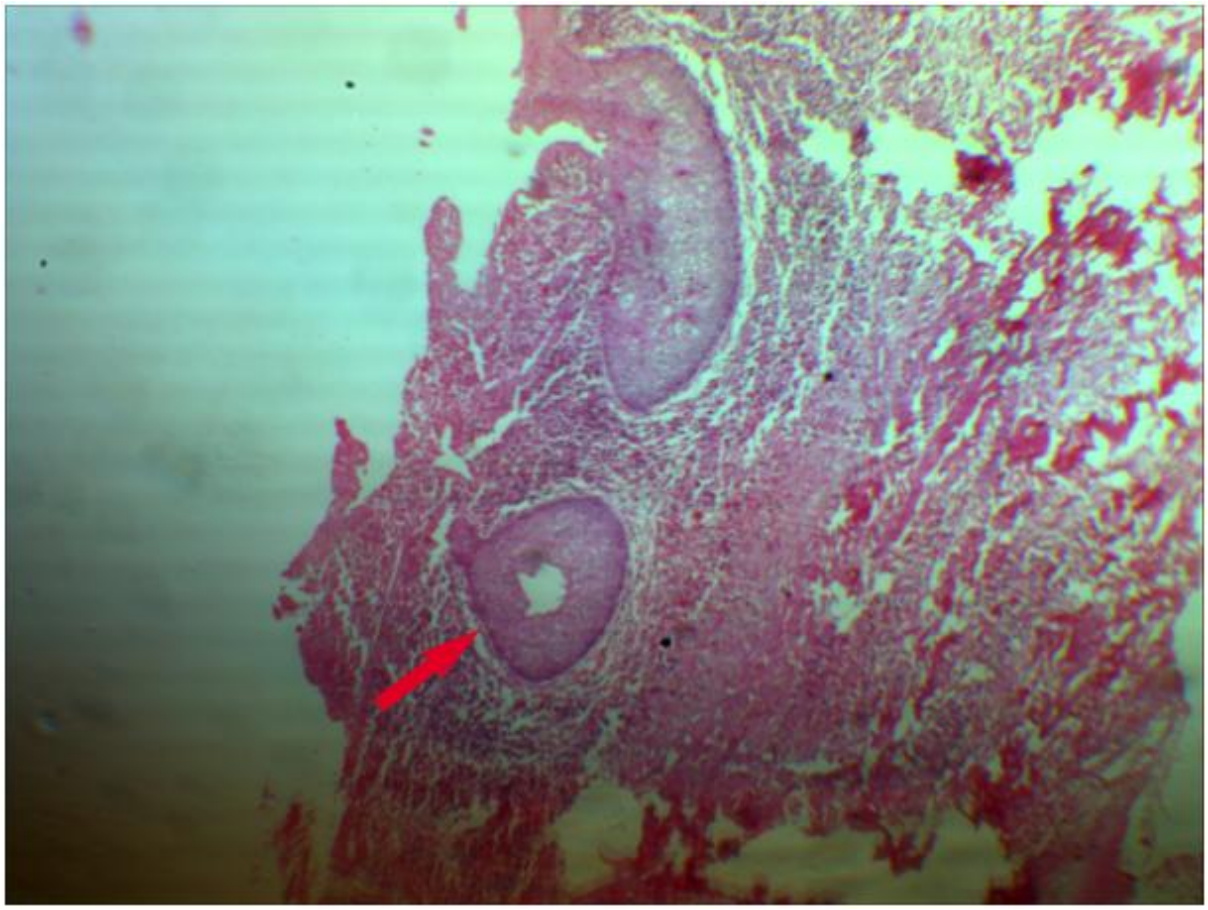
Fig(10):photomicrograph of histopathological section of OKC showing area of thin ortho keratiaized epithelium lining (Orange arrow) and some area of thick lining (Green arrow). Note the detached of epithelium lining(Blue arrow)(H&E x 100).



Fig(11): photomicrograph of histopathological section of OKC showing cyst cavity containing Keratin squamous (Green arrow) (H&E x 100).

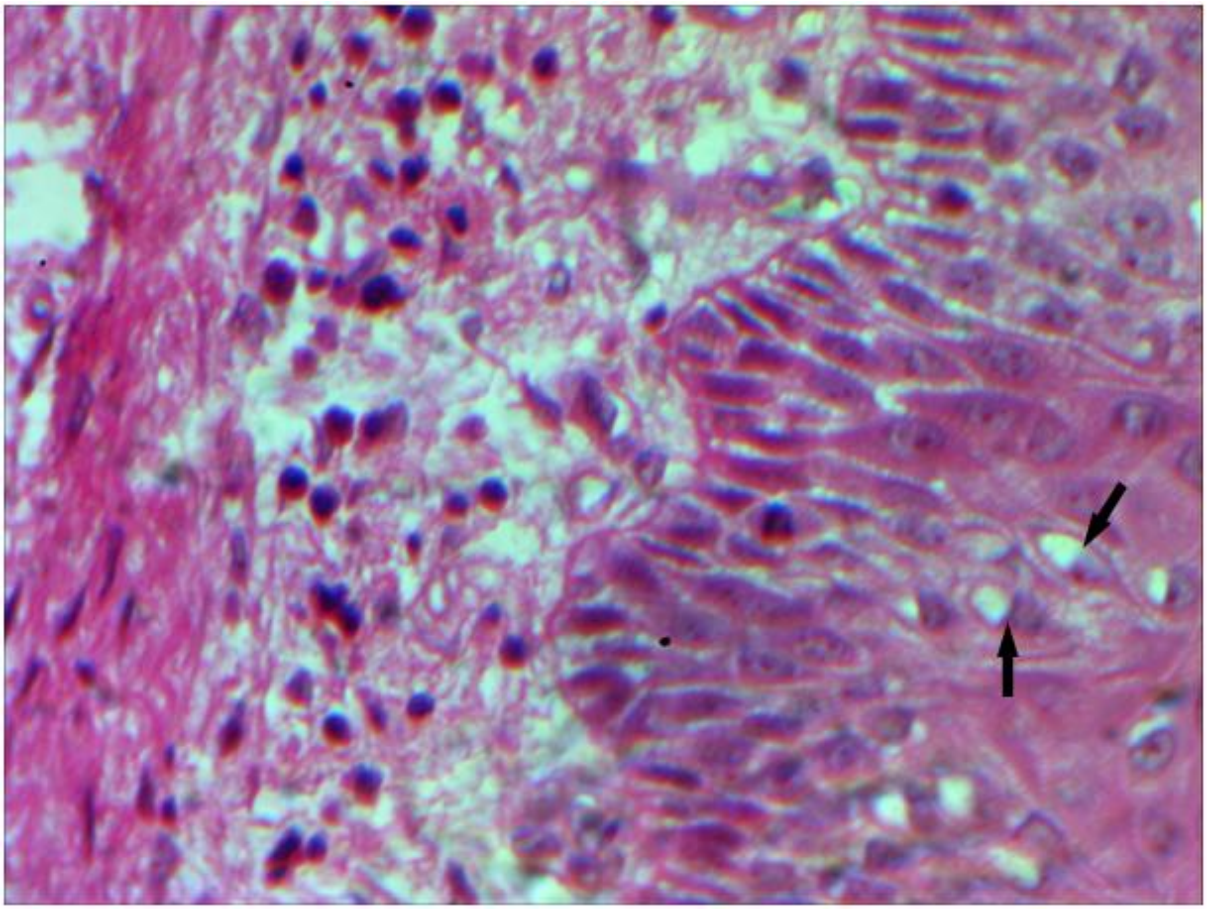


Fig(12): photomicrograph of histopathological section of OKC showing Daughter cyst (red arrow) (H&E x 100)



Fig(13): photomicrograph of histopathological section of OKC showing doughter cyst(red arrow) (H&E x 40)

Fig(14):photomicrograph of histopathological section of OKC shows cyst wall contains satellite cyst (red arrow) (H&E x 40).



Fig(15):photomicrograph of histopathological section of OKC shows mitotic cells at basal cell area of the lining epithelium (Black arrows) (H&E x 400).

The Immune Reaction of the BCL-2 Gene in the Odontogenic Keratocyst

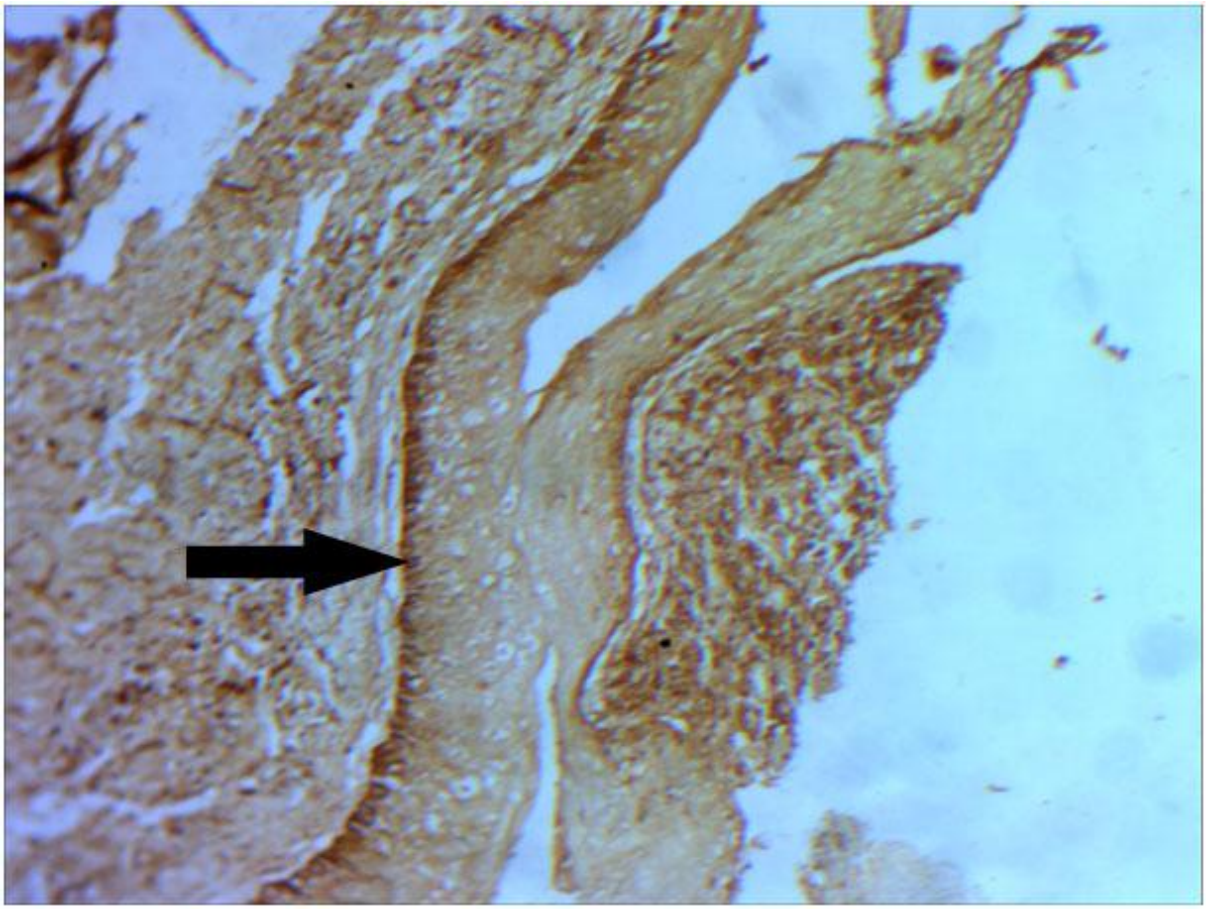
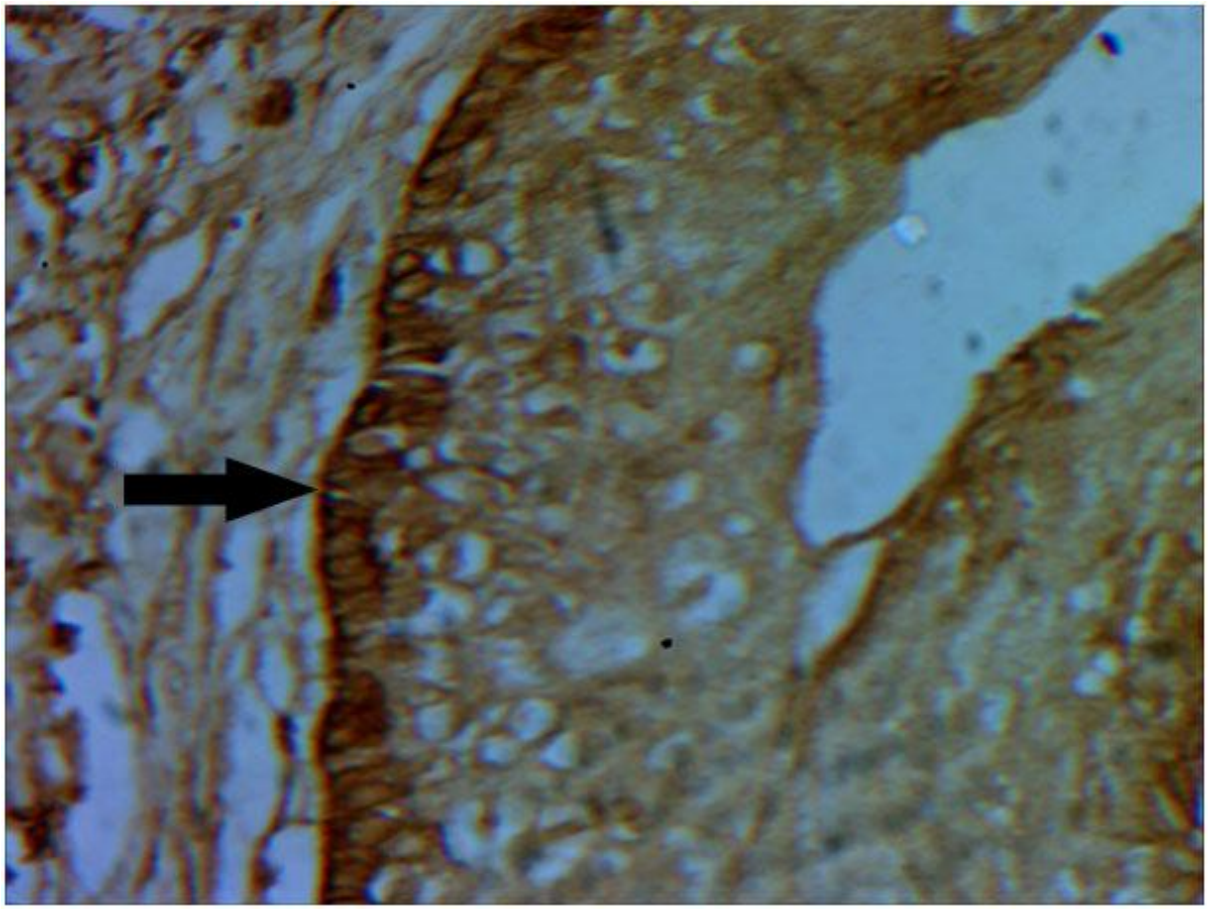


Figure (16): Photomicrograph of Immune histochemical section of OKC showing moderate to intense positive reaction in basal cell layer to BCL-2 (Black arrow) (BCL-2 immune–stain x 100)



Figure(17): High power view of the previous case basal immune reaction (Black arrow) (BCL2 immune stain. x 400).

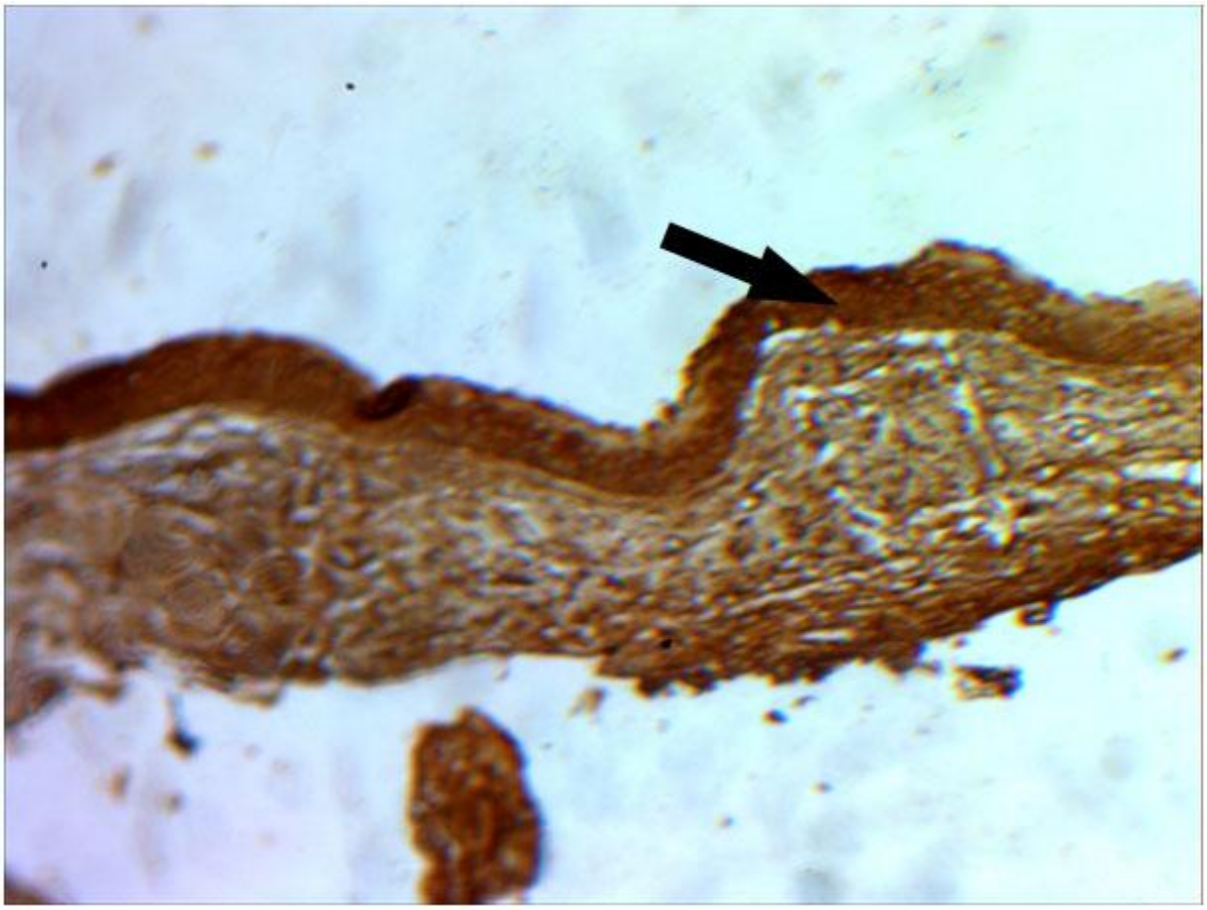
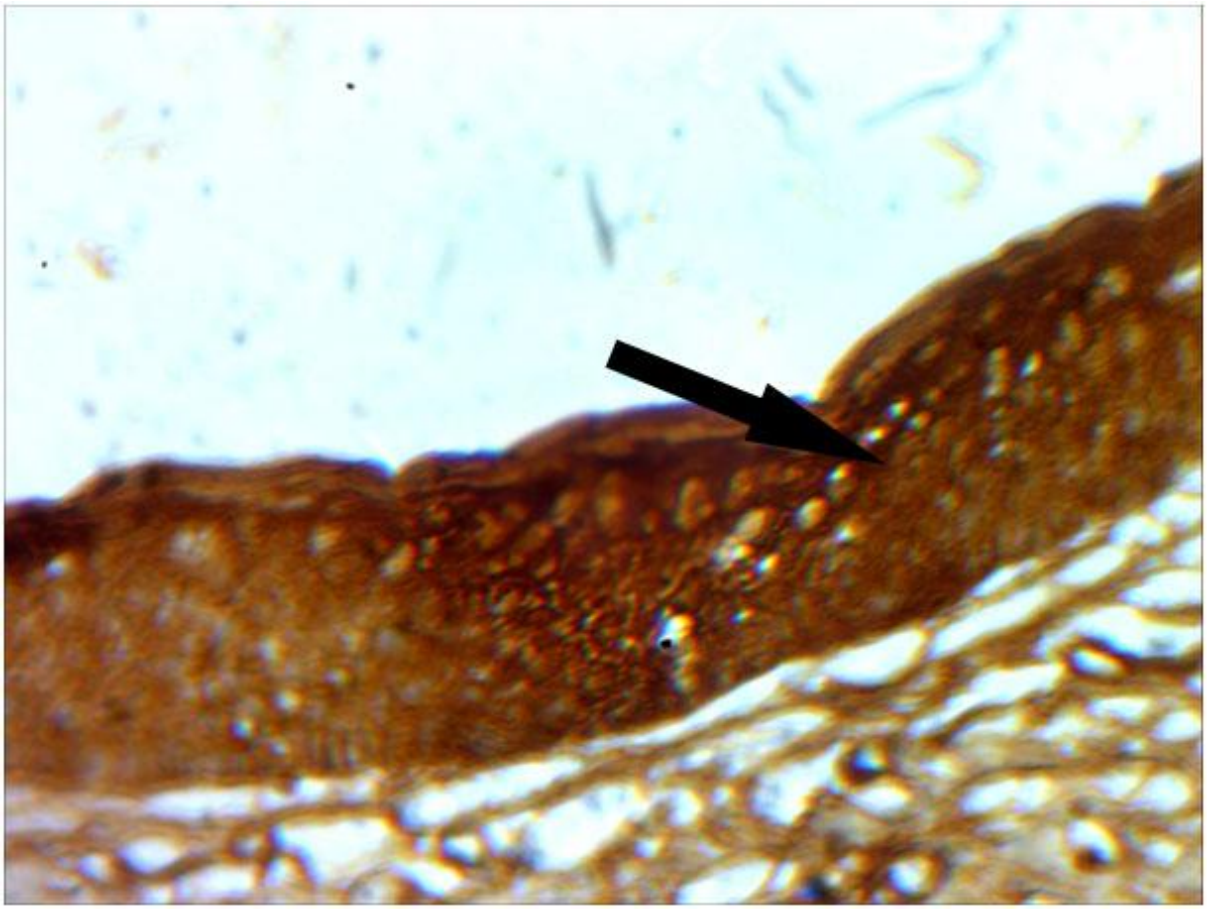
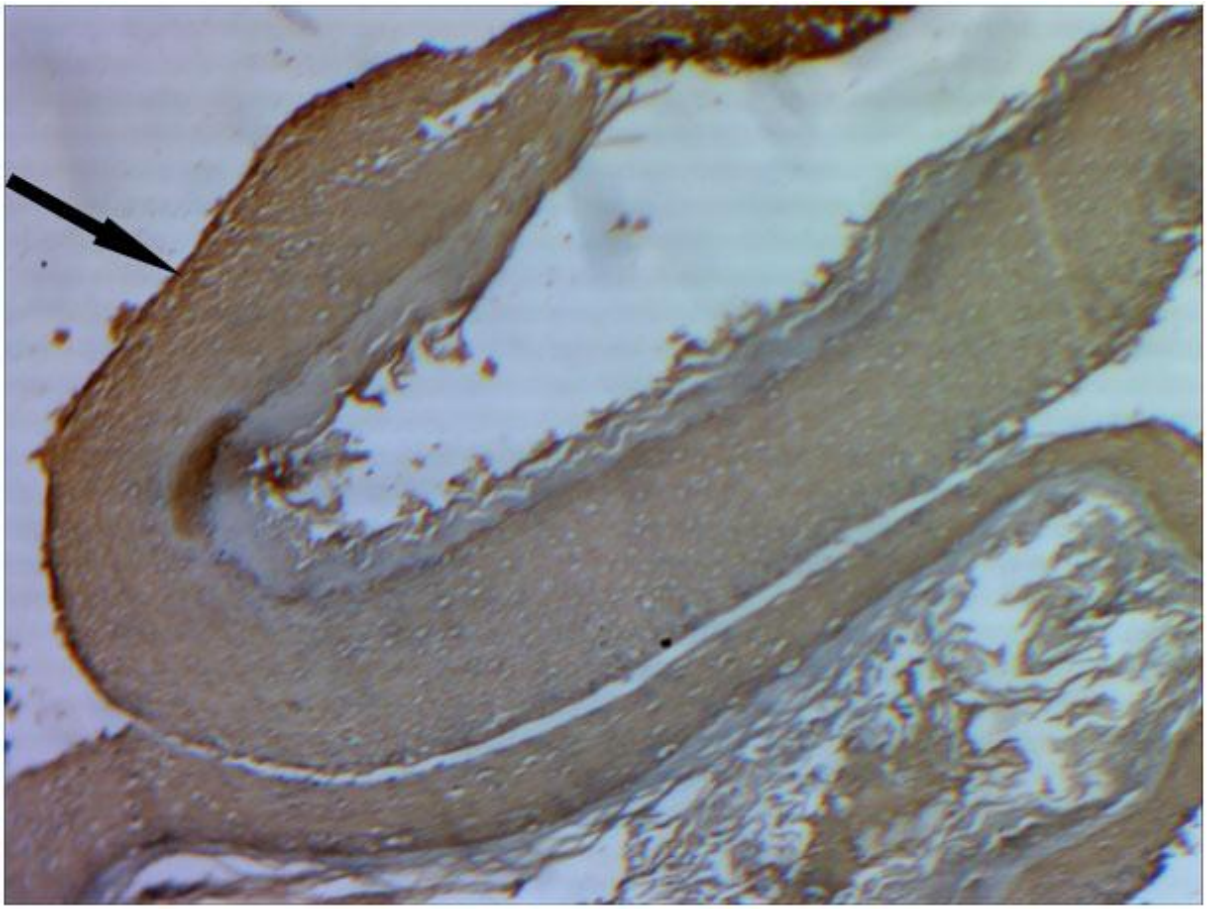


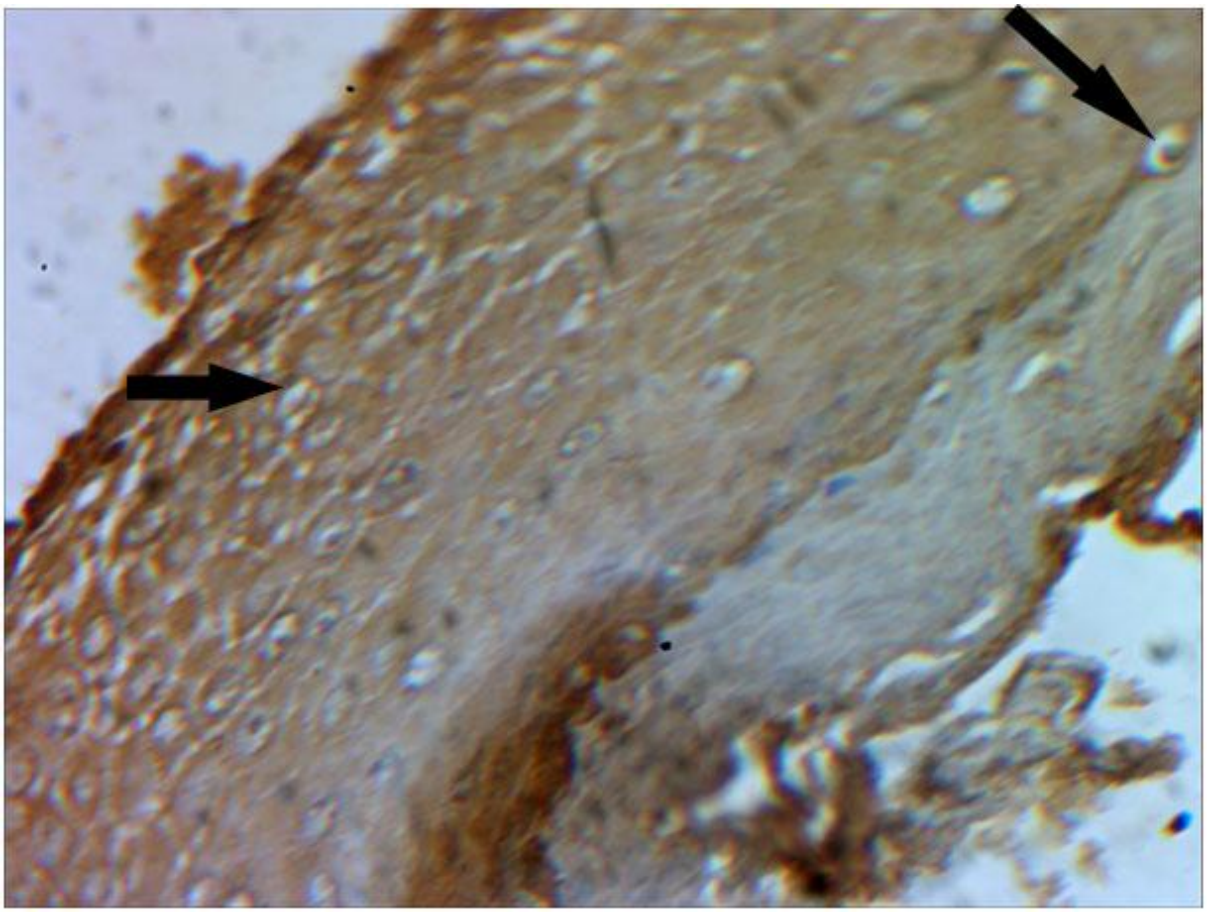
Figure (18): Photomicrograph of histochemical section of OKC showing intense diffuse positive reaction in basal and supra basal cell layer to BCL-2 combined cytoplasm and nuclear reaction (Black arrow) (BCL-2 immune–stain x 100)



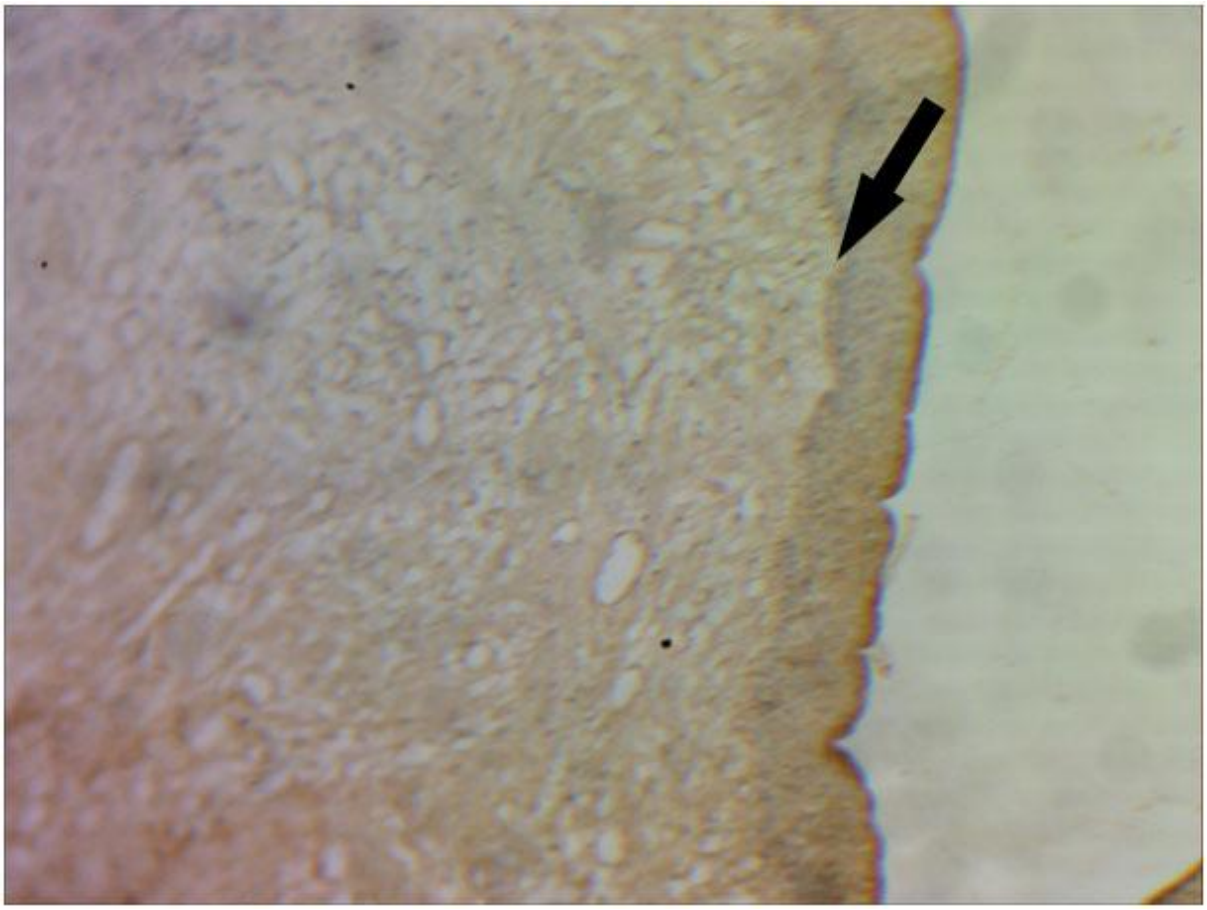
Figure(19): High power view of the previous case basal and supra basal immune reaction (Black arrow) (BCL2 immune stain. x 400).



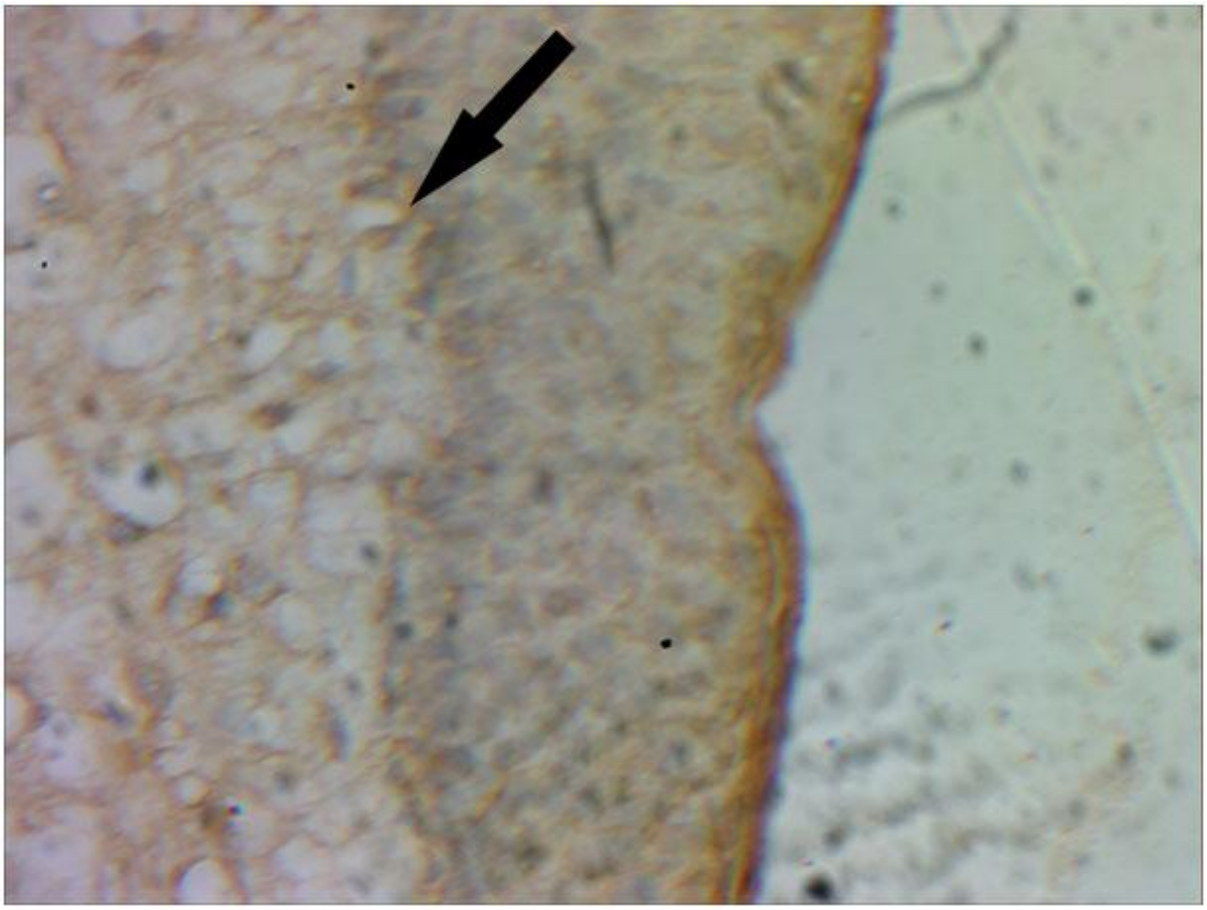
Figure(20): Photomicrograph of histochemical section of OKC showing mild basal reaction (Black arrow) (BCL2 immune stain. x 100)



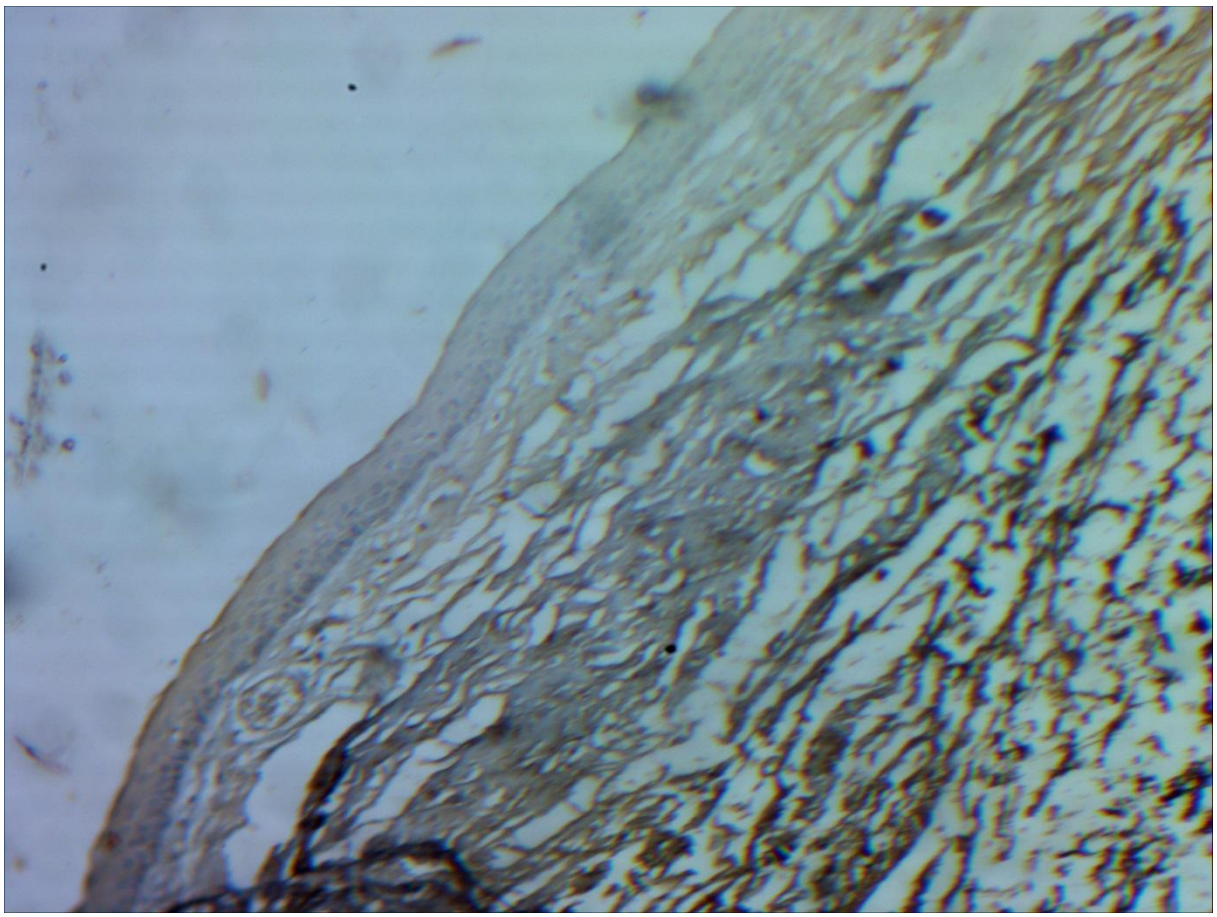
Figure(21): Photomicrograph of histochemical section of OKC shows mild to moderate reaction to BCl2 involved nuclear in the basal cell layer (Black arrow) (BCL2 immune stain. x 400)



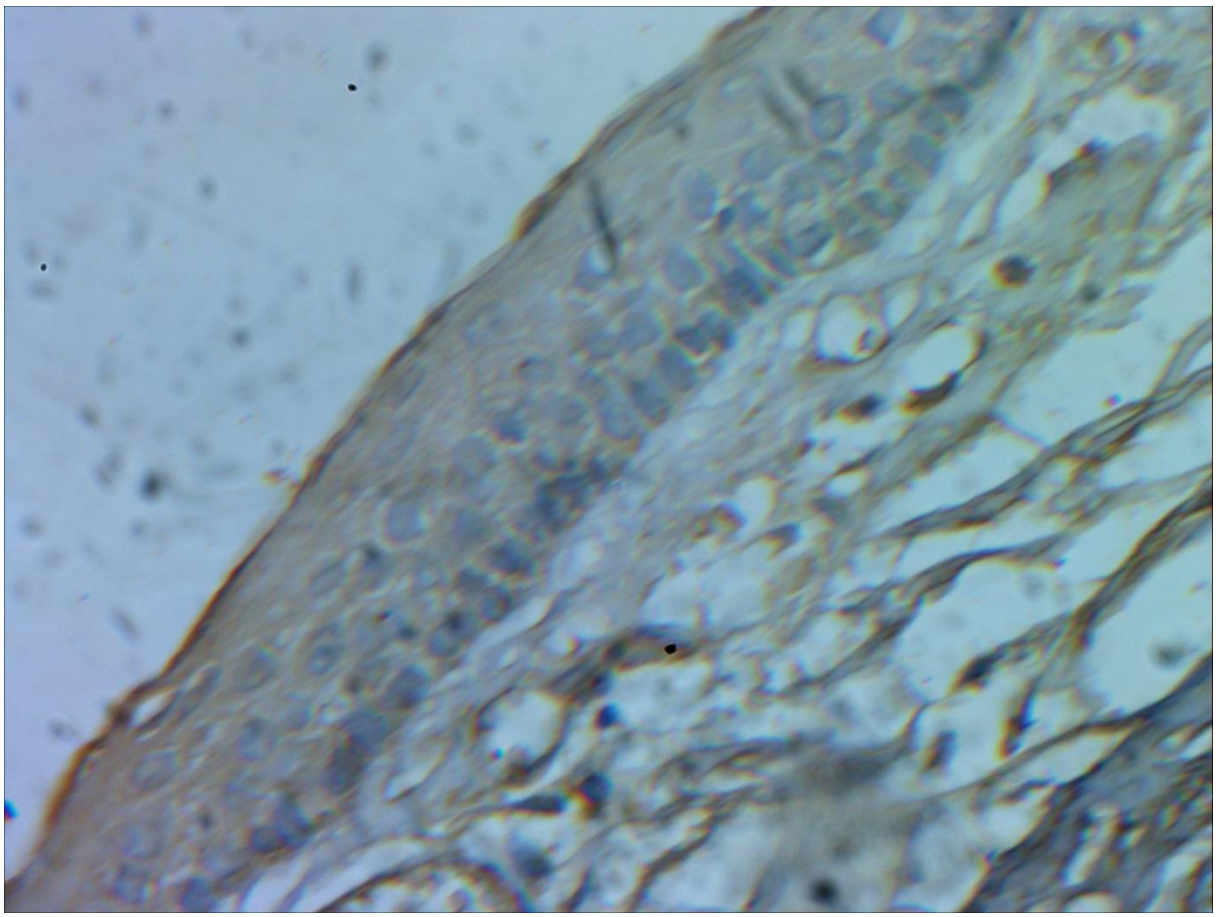
Figure(22): Photomicrograph of histochemical section of OKC shows very weak reaction (Black arrow) (BCL2 immune stain. x 100)



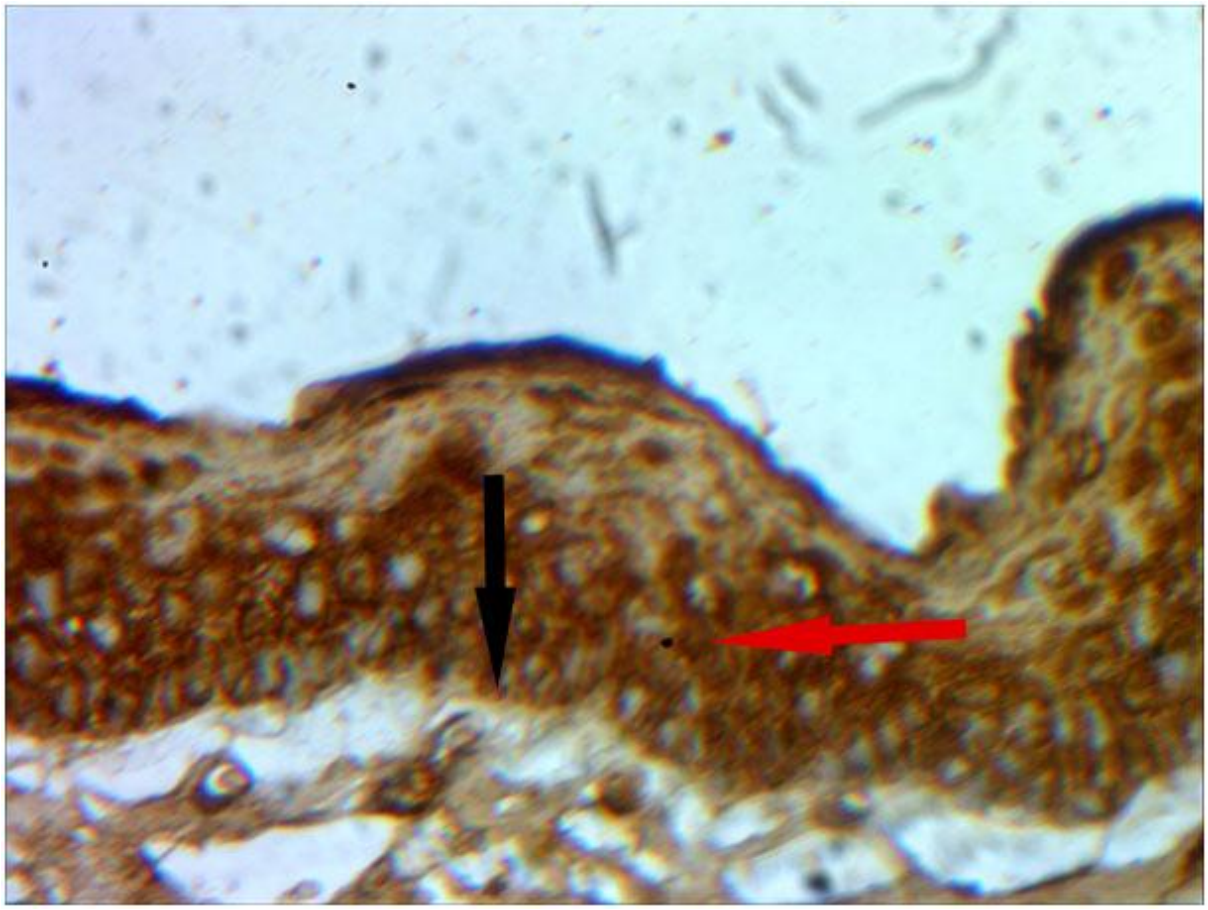
Figure(23): Photomicrograph of histochemical section of OKC of the previous case shows very weak reaction (Black arrow) (BCL2 immune stain. x 400)



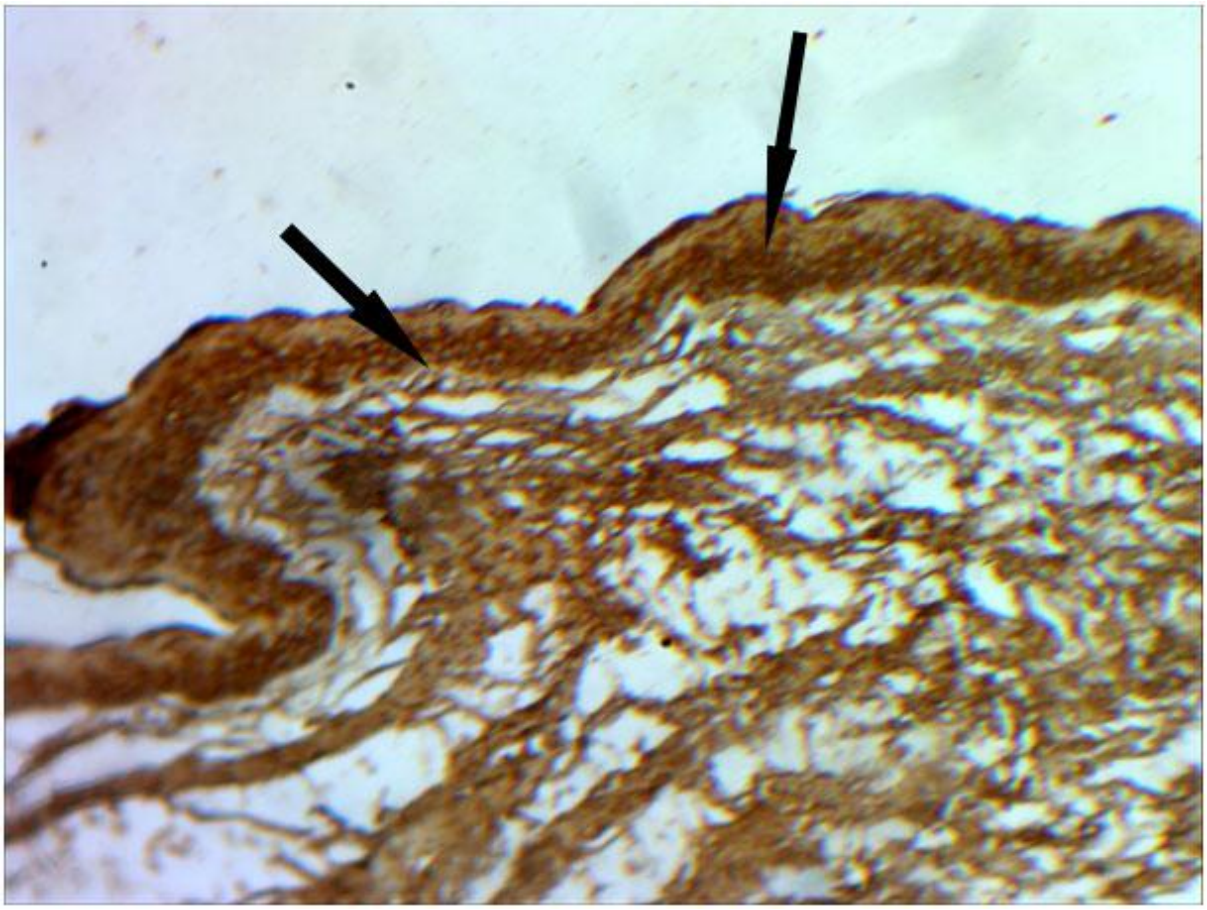
Figure(24): Photomicrograph of histochemical section of OKC shows negative reaction (BCL2 immune stain. x 100)



Figure(25): Photomicrograph of histochemical section of OKC shows negative reaction (BCL2 immune stain. x 400).



Figure(26): Photomicrograph of histochemical section of OKC shows Intense reaction at basal (Black arrow) and supra basal layers involve nuclear, cytoplasmic (red arrow) (BCL2 immune stain. x 400).



Figure(27): Photomicrograph of histochemical section of OKC shows Intense reaction at basal and supra basal layers involve nuclear, cytoplasmic (Black arrows) (BCL2 immune stain. x 100).

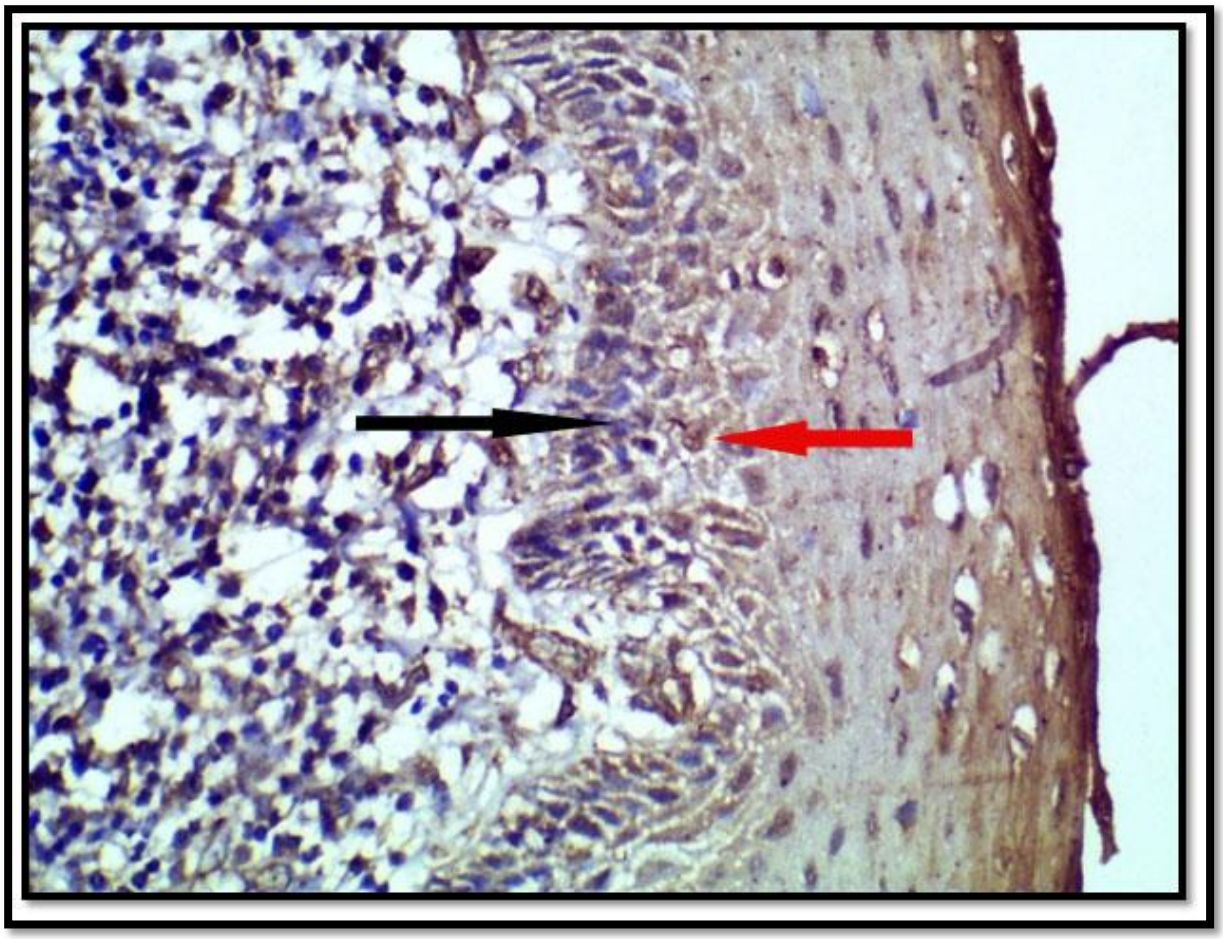


Fig (28) : Photomicrograph of histochemical section of odontogenic kerato cyst shows positive reaction at basal (Black arrow) and supra basal layer (red arrow) to BCl2 mainly nuclear reaction (anti apoptosis protein) (BCL2 immune stain. x 400).

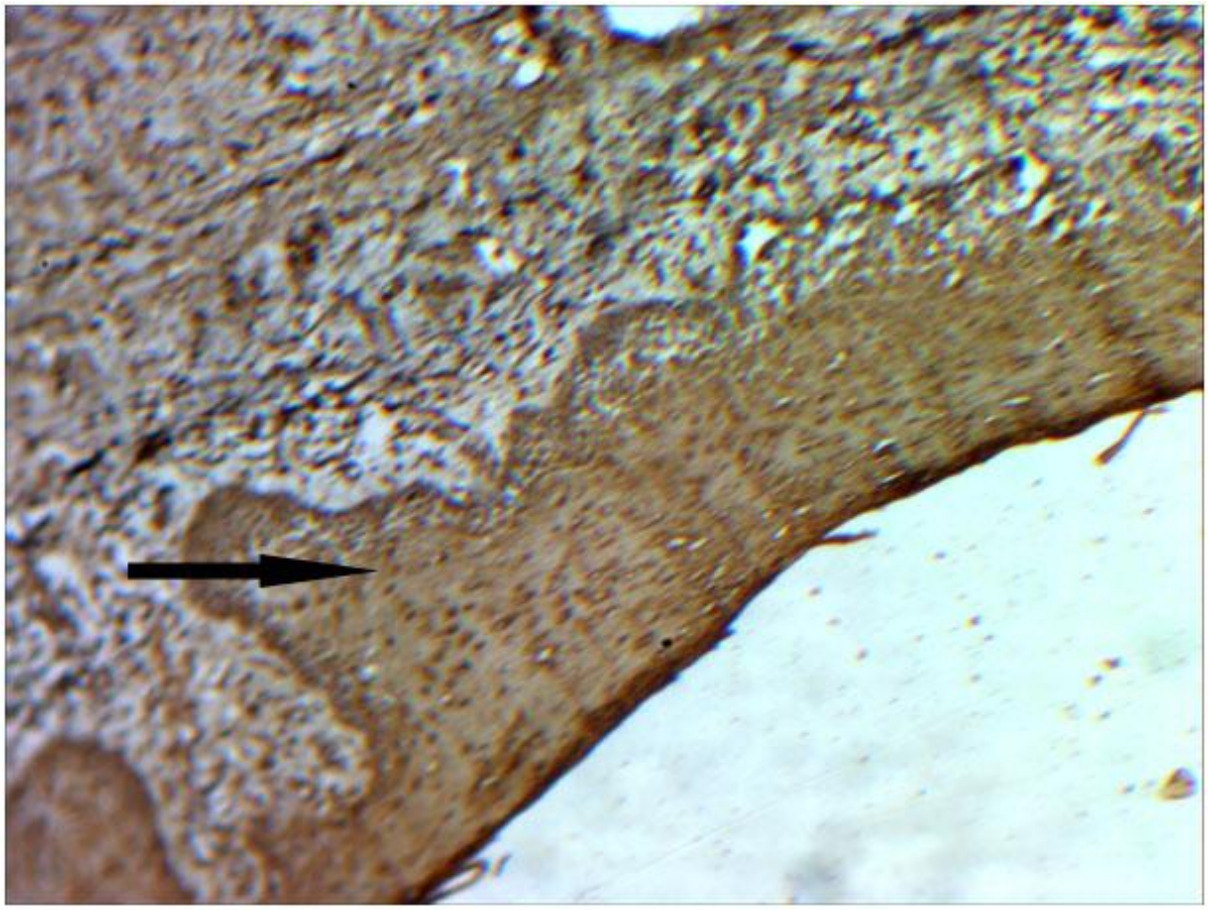
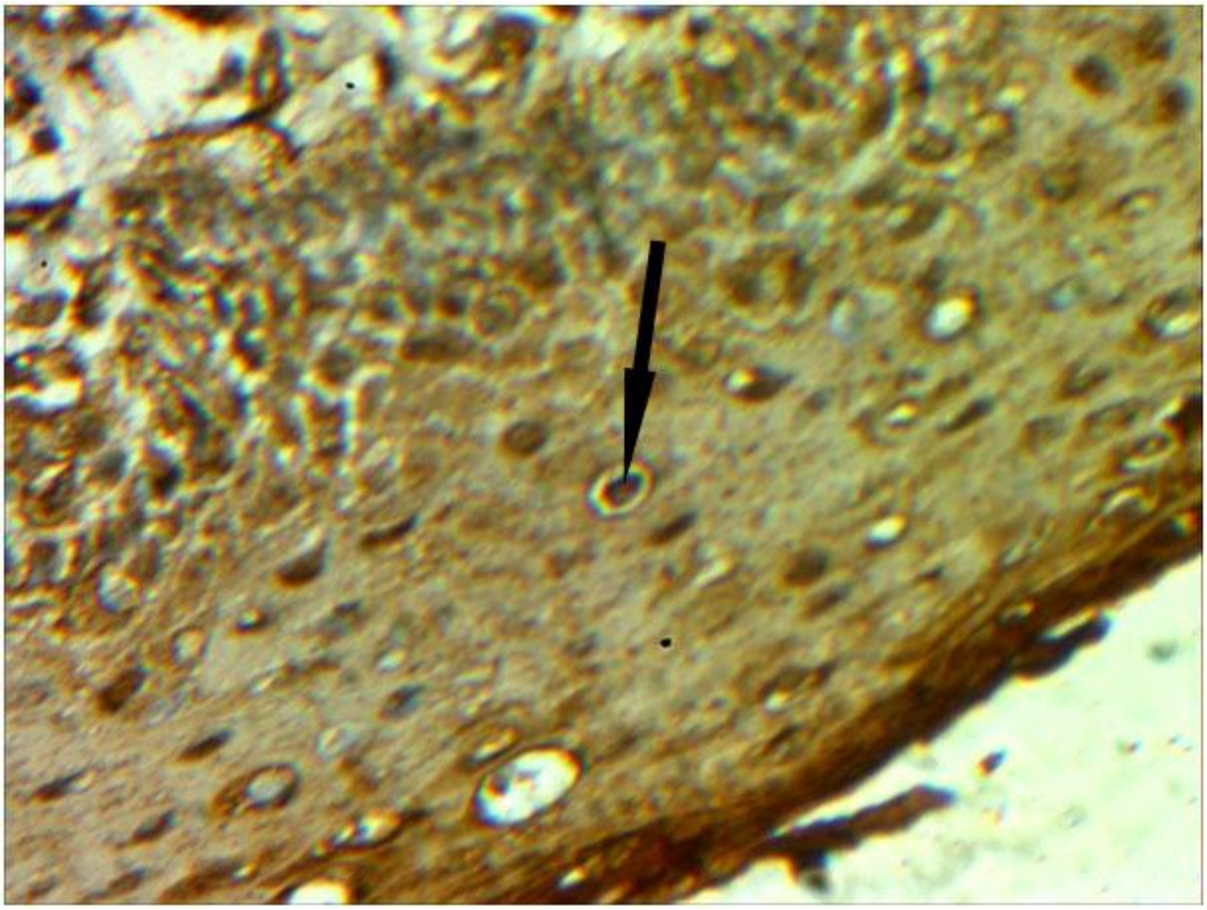


Fig (29) : Photomicrograph of histochemical section of odontogenic kerato cyst shows hyper plastic epithelium (Black arrow) with positive moderate reaction at basal and supra basal layer to BCl2(anti apoptosis protein) (BCL2 immune stain. x 100).



Fig(30): Photomicrograph of histochemical section of odontogenic kerato cyst shows nuclear positive reaction (Black arrow) (BCL2 immune stain. x 400).

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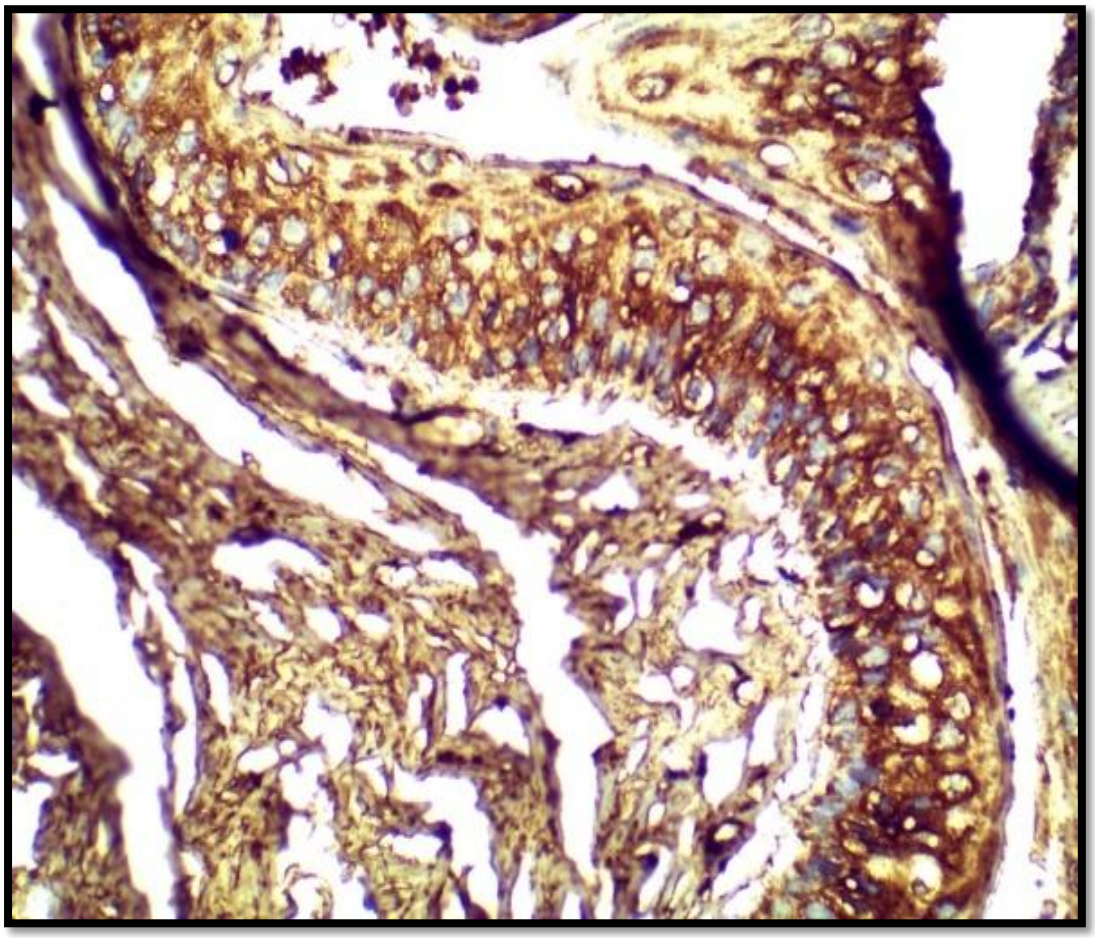
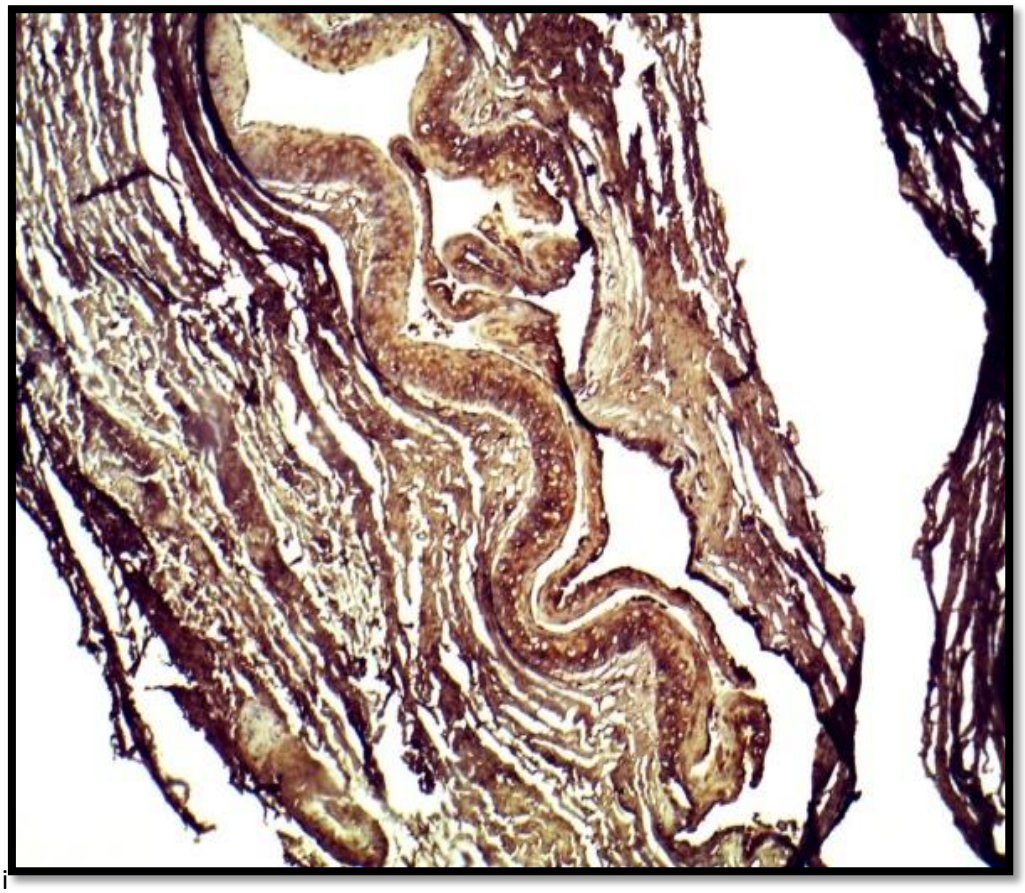


Fig(31): Photomicrograph of histochemical section of odontogenic kerato cyst shows nuclear , cytoplasmic positive reaction (BCL2 immune stain. x 400).



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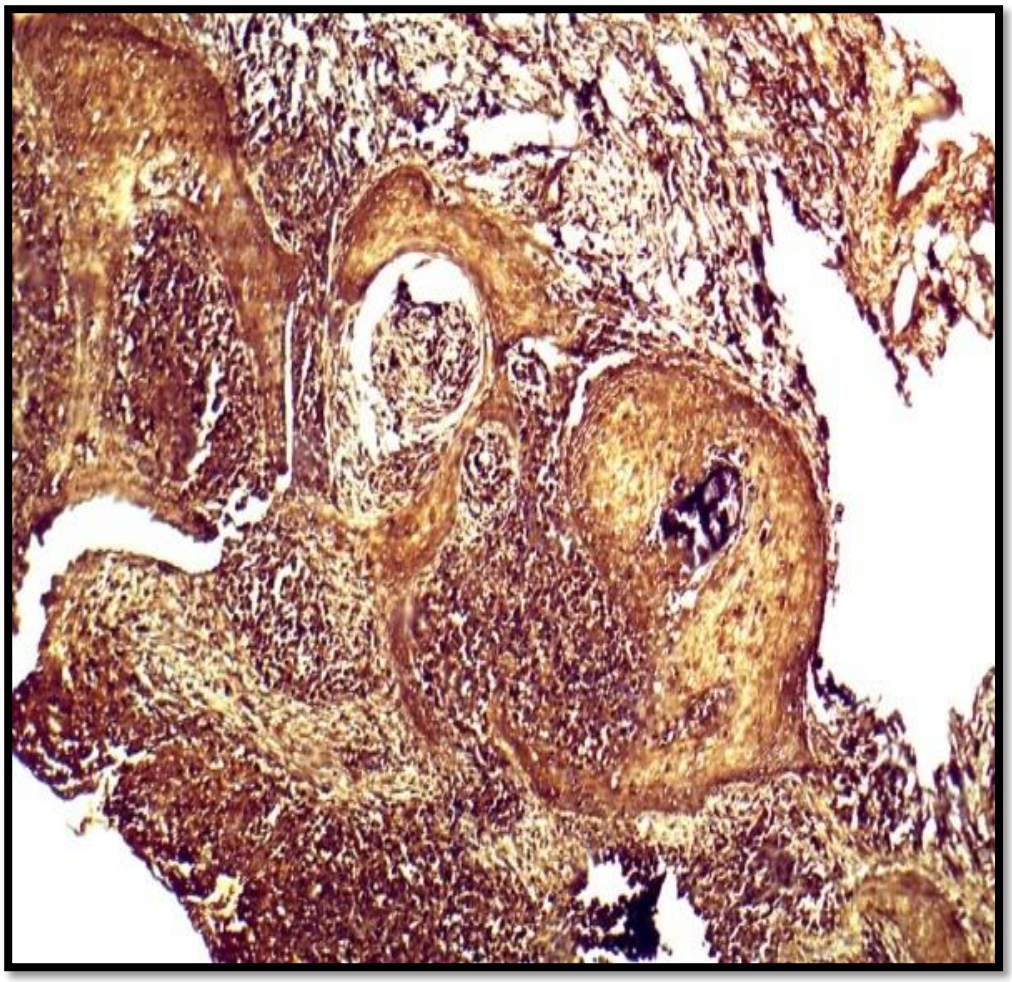
Fig(32):Photomicrograph of a histochemical section of Odontogenic kerato cyst demonstrates strong brownish staining of the intense reaction for Bcl-2(immune stain×100), diffuse staining.



Fig(33):Photomicrograph of a histochemical section of Odontogenic kerato cyst demonstrates positive staining cell (brown)in immune histochemical stain for anti apoptosis protein BCL-2 mainly nuclear reaction (immune stain. x 100).

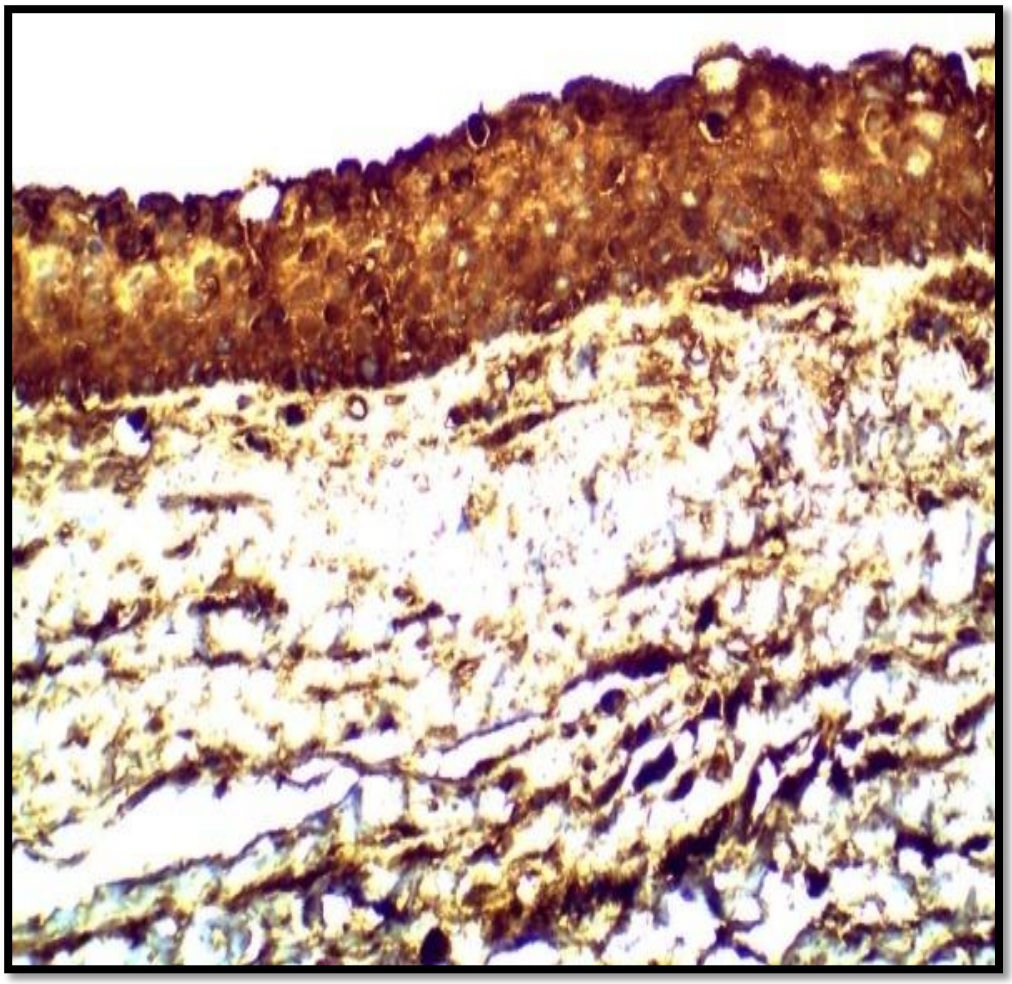
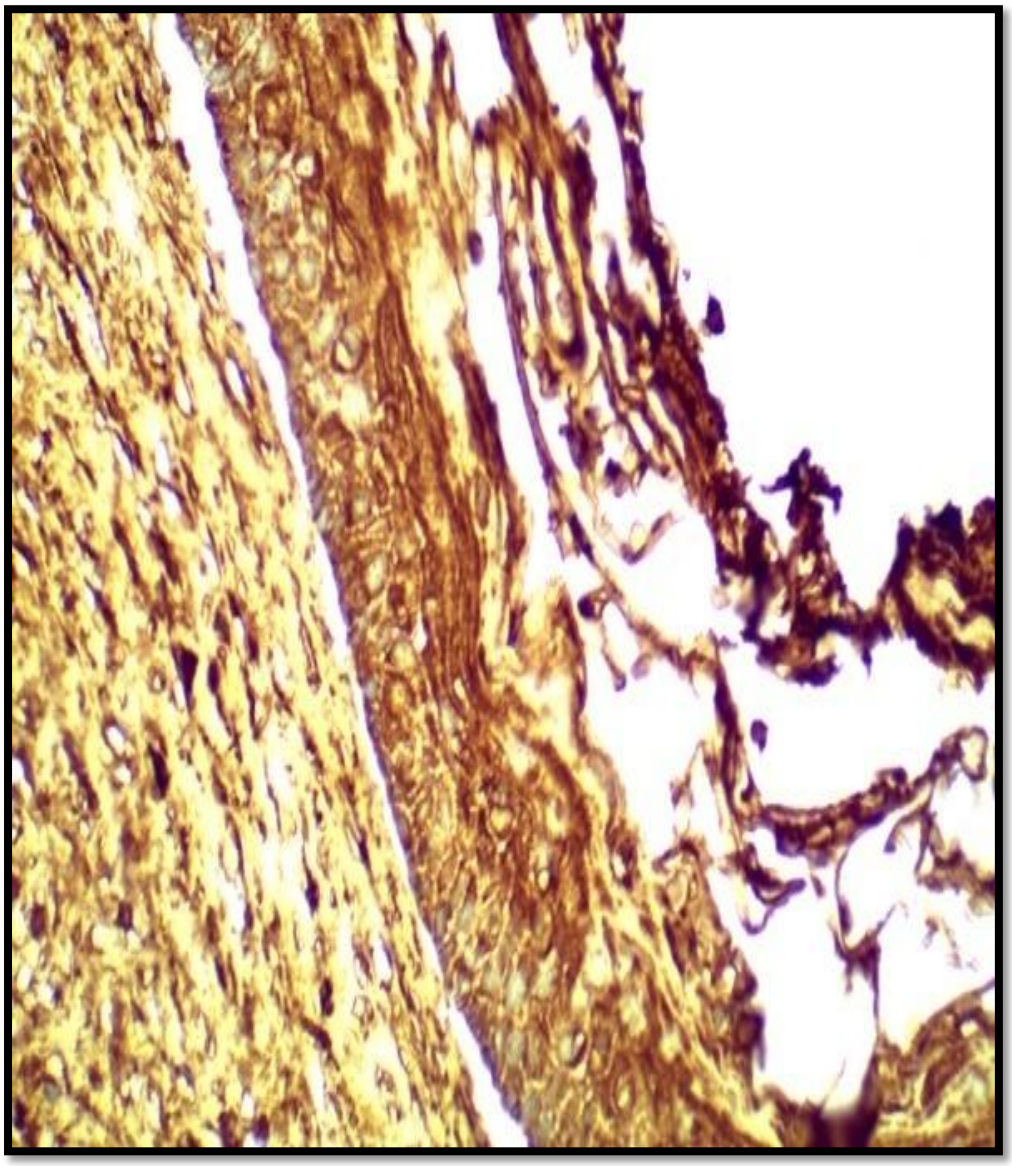
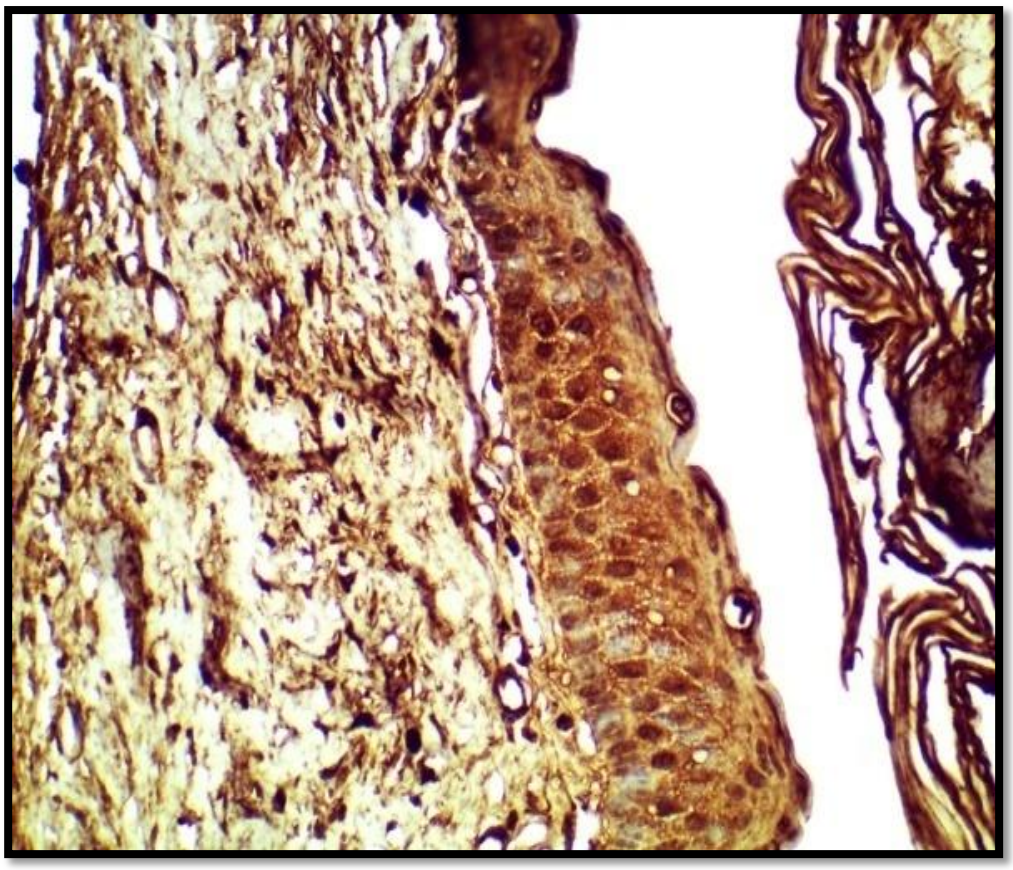


Fig (34): Photomicrograph of a histochemical section of Odontogenic kerato cyst shows intense reaction in both basal and supra basal layer involve nuclear cytoplasmic (BCL2 immune stain. x 400).



Fig(35): Odontogenic kerato cyst notes positive moderate nuclear reaction for BCl-2. (immune stain. x 100).



Fig(36): Photomicrograph of a histochemical section of Odontogenic kerato cyst shows intense reaction mainly nuclear in both basal and supra basal (BCl2 immune stain. x 400).

Chapter 6

DISSCUSSION

6. Discussion

The study conducted in the faculty of dentistry, university of Benghazi, this retrospective study duration was from Jan 1997 to December 2014,in our study the nationality of OKCs cases were Libyan and residency all from Benghazi except one case from Tobruk, a M:F ratio 1.9: 1 Most of cases were found in the second to third decades While Duangrudee etal in 2006[3],showed that Male: Female ratio was1:1.2 ,the average age at the time of presentation was 33.25 years with arrange from 18 to 65 years.

Most of the cases were found in the second to fourth decades, the age distribution in our study was quite similar to those of most other studies revealed that the peak incidence between 11 and 40 years of age and rare occurrence in individuals older than age of 70. OKCs were reported as having a male predilection in most other studies, the lesions of OKCs were more commonly found in males than females [31,32].

Orthokeratinized Odontogenic Cyst (OOC) is a developmental cyst with which is more common in the 4th decade of life, males are more frequently affected then females[33],our results consistent with Richard study in 2009[12], results in which OKCs tend to affect younger adults with peak ages in the second and third decades [12],more over Norio study in 2009 revealed that there was an even predilection with a male :female ratio of 1:1. More than three–quarter of subject**s** were <60 years old at the time of surgery (median 40 years; range 15–72 years),median follow-up period was 33 months. Nearly one- third of subjects had a recurrence those on the maxilla, one was in anterior maxillary region, and two were in posterior maxillary zone[34].

Regarding gender distribution, our findings were in accordance with those of other studies, which present male predominance as well as the age peaks were 2nd and 4th decades, as was registered by Leite and etal 2001[31], study in

2011.However, most other studies indicate peaks during 2nd and 3rd decades also Odontogenic Keratocystic tumor(OKT) classification according to WHO reported that the tumor affected both maxilla and mandible simultaneously,the others affected the mandible alone most frequently[35],our study recurrence was zero this similar to China study recurrence was zero[36].

Also in our study all cysts are single no multiple cysts or associated Gorlin – Goltz syndrome, this was in consistent with An and his colleagues study which reported that the solitary OKC seems to be less biologically aggressive and should be classified as a cyst rather than a tumor, means that at least few of OKCs manifests as ordinary cysts**.** Some of the study findings could support the theory that OKCs are with high proliferative, probably that these lesions are developmental cysts with some neoplastic properties because of the high intrinsic growth potential, WHO recommends the term KCOT as it better reflects the neoplastic nature of the lesion; however, this reclassification has not yet been universally accepted[19].

The sites of the OKCs in this study the majority of cases were found in the mandible, followed by maxilla and rare in other places such as cheek and palate similar finding in Duangrudee etal[3] ,study in which most of subjects of OKCs were found the most common sites were in the mandibular body, angle, and ramus regions maxilla and minority of lesions involved more than area[3], these results are similar to the data reported that the majority of lesions were located in the mandible and the remaining were seen in the maxilla Most bone-perforating lesions were localized buccally this is quite similar to the result from other studies , OKCs occurred more frequently in the mandible than the maxilla[32, 37], also the OKCs occurred more frequently in the mandible than maxilla this is quite similar results from our study [38].

Furthermore, Same results demonstrated that OKCs were most commonly found in the mandible and about 50% are found at the angle, often associated with the crown of an unerupted third molar [12], odontogenickeratocyst (OKC) is defined in the recent WHO classification as a cystic tumor lined by parakeratotic squamous epithelium and designated as keratocysticodontogenictumor[39].

In our study the pathological examination of cyst lining showed that most of the cyst is lined by a thin 80% , regular stratified epithelium and had thick stratified squamous epithelium in only 20% of lesions and composed of prominent basal layer that shows more than half of lesions (55%) were palisaded and 45% not palisaded, with intercellular separation of the suprabasal cells to resemble the normal satellite and stratified squamous epithelium,further more, we found the majority of the cysts were surface layer are parakeratinized represent 75 % while surface layer are orthokeratinized in 25 % of the lesions.

Also the histological examination found that the mitotic figure are basal in 80% of the patients and seen supra basal in 20 % of the lesions and relationship between. Parakeratinized and positive immune reactions statistically significant , while orthokeratinized in relation to immune positive reactions OKC statistically non significant, RichardLikewise,Duangrudee study(2009) recorded almost all of the lesions were diagnosed histologically as parakeratinized-odontogenickeratocysts,In addition, many researches, clinical trials and projects in different geographical and settings have confirmed that the parakeratinized and orthokeratinized OKCs were significantly different in molecular area as well as the recurrence rate orthokeratinized OKCs had a lower recurrence rate than the parakeratinized OKCs study[40].

Donff et al(1972)[41],demonstrated that the collagenase activity in keratocyst epithelium which appears to relate to the ability of keratocysts to grow

expansively within bone, this could explain why the majority of patients had the symptoms less than 3 months[41].

In this study concerning the positive results histological finding of cyst wall were detected in lesions, the inflammatory mechanisms in this study demonstrated that few inflammatory cells of cell wall represents 75% of lesions and presence of satellite cyst 40% of the lesions as well as epithelial residues were constitute 40% of the lesions Presence of inflammatory cells in positive immune reactions OKC were statistically significant.

Whereas**,** the content of the cavity in the present study were Kerattine squamous were prominent in 60% of the cyst cavity and absent in 40% of the cavity of the lesions.Similarly Kerattine squamous, the epithelial lining is folded and may appear to extend in long finger-like processes, many cysts show small islands of odontogenic epithelium in the wall and there may be budding of the basal layers, satellite cysts are also encountered, sometimes epithelial islands and satellite cysts are so prominent that they produce a multicystic or solid variant[42].

From literature, the content of cystic cavity presented as either straw color fluid or keratin curd with a ratio of 1:7.4 (straw color fluid: keratin curd),straw color fluid content was not found in the patient who presented with signs of infection[3], furthermore, recorded accumulation in the cystic fluid of odontogenickeratocysts dentigerous cysts, and radicular cysts of serum proteins from the vasculature have been thought to elevate the hydrostatic pressure and maintain their expansion[43].

In addition, a study to analyze the Bcl-2 protein expression in the epithelial lining of the study contained 45 samples of which:15 Odontogenickeratocyst (OKC), 15Radicular (RC), and15Dentigerous cysts (DC) confirmed that OKCs develop from the odontogenic epithelium or it's remnants[44], it is well known

that the OKCs shown aggressive behavior, recurring at greater frequency than other types of odontogenic cysts,the clinical findings have been supported by numerous reports focusing on the greater proliferative potential of the epithelial lining of OKCs compared with other types of odontogenic cysts[44].

Furthermore, this is in line with the results of Simirina in Pakistani who showed that the histologically the WHO (1992) classified the OKC into three variants parakeratinized, orthokeratinized and combination of the two,the parakeratinized type of OKC was associated with nevoid basal cell carcinoma syndrome[24], the parakeratinized lining of the parakeratinized variant of OKC is of uniform thickness which is 5-8 cells thick, dysplastic changes are seen in this layer, the basal cell layer has cuboidal cells which lack rete ridges, the cyst lining has buddings and is much folded, satellite cysts are present in the wall, inflammatory cells are typically absent or scanty and inflammatory conditions the fibrous capsule thickens cause ulceration and keratinization disappears [24].

Orthokeratinized Odontogenic Cyst (OOC) is a developmental cyst with which orthokeratinized variant shows orthkeratinization, a squamous basal layer, a granular layer and keratin present in the cyst[33],our finding of the subjects were positive reactions in the majority of samples (15 out of 20) all were diffuse where as only 5 out of 20 were negative reactions,among the positive reactions there were cysts (46.7 %) are intense reactions to Bcl 2 expression, the majority of the OKCs were intense, diffuse, the immune histochemical findings that deserves attention is the occurrence of positive reactions for bcl-2 expression (protein anti apoptosis), present in 75% of the lesions were classified into three categories weak , moderate and intense reactions in 20 %, 33.3 % & 46.7 % respectively .

According to involvement there were 11 cases (73.33%) nuclear and 4 cases (26.67%) combined nuclear and cytoplasmic, when compared with Jahanshah-iresults (11 of the 19) stained OKCs, positively stained cells were observed in the basal layer while in the other Bcl-2 positive OKCs(8 of 19), the stained cells were in the basal/supra basal region, in Bcl-2 positive radicular and dentigerous cysts, positive cells were located in the basal / suprabasal layers, no statistically significant relationship was found between the intensity of inflammation and Bcl-2 staining in positively-stained OKCs (Spearman = 0.06, P= 0.8). The sensitivity, specificity and positive and negative predictive values of Bcl-2 staining for the differentiation of OKC from the other studied cysts were 95%, 90%, 90% and 97%respectively, with acceptable inter-observer agreement (Kappa = 0.85)[20].

On the other hand, the histological appearances are characteristic in recent studies in which the cyst is lined by a thin, regular stratified epithelium showing a finely corrugated parakeratinized surface,the epithelial lining is folded and may appear to extend in long finger-like processes, many cysts show small islands of odontogenic epithelium in the wall and there may be budding of the basal layers, satellites cysts are also encountered and sometimes epithelial islands and satellite cysts are so prominent that they produce a multicystic or solid variant[12,42].

The finding are similar to those in previous studies reported that Bcl-2 positive cells are predominantly located basally, thus supporting the concept that apoptosis does not occur in the basal cells of the lining epithelium. TUNEL-positive cells have been detected in the surface layer of KCOTs, indicating marked levels of apoptosis[10],thus, Bcl-2 inhibits apoptosis to facilitate cellular proliferation in the basal and suprabasal layers, whereas apoptosis maintains the

homeostasis of the thickness of the lining epithelium and allows the synthesis of large amounts of keratin in the surface layer of KCOTs [10].

Chapter 7

SUMMARY AND CONCLUSION

7. Summary and Conclusion

In general our results concluded that OKCs were solitary, no recurrence, the majority of OKC seems to be biologically aggressive and should be classified as a tumor rather than a cyst, because the majority of OKCs were with high proliferative, probably that lesions are developmental cysts with some neoplastic properties because of the high intrinsic growth potential.

The histopathological appearance of OKCS firstly, characterized by thin stratified squamous epithelium in the majority of cases,most of OKCs showed a palisaded basal cell layer, the surface layer of cyst lining were Parakeratinized and only few were Orthokeratinized also numerous and supra basal cell, secondly, cell wall contained the following: -

1. Inflammatory cell were prominent in the underlying connective tissue15 out of 20 (75%) of OKC subjects .
2. Satellite cysts were partly observed that they produce solid variant in 40% of Subjects.
3. Keratin squames filled the cyst cavity in 12 out of 20 (60%) of Subjects.
4. Presence of epithelial residues also in 40% of lesions.

Regarding immunohistochemical, we concluded that the number of Bcl-2 expression (protein anti apopotosis) positive cells was high, Bcl-2 expression was observed in 15 OKCs (75%), the majority of the cysts are positive stained with intense reactions and diffuse reactions, a basal/supra basal distribution of Bcl-2 positive cells was seen in some odontogenickeratocysts which may have a significant impact on the behavior of this cyst Immuno reactivity was mainly observed in both basal and supra basal layers, in our study 3 of the 20 stained

OKCs, positively stained cells were observed in the basal layer, while in the other Bcl-2positive OKCs(12 of 20), the stained cells were in the basal/supra basal region.

Finally, we concluded that OKCs were characterized by higher expression of BCl-2 in basal cell epithelium which indicate to antiapoptotic activity of BCl2 in lining epithelium which lead to cell survival and formation of tumor , this result can explain why OKC is reclassified to KCOT and explain the aggressive behavior of this lesion

Chapter 8

RECOMMENDATIONS

8. RECOMMENDATIONS

We recommend to complete the medical files in the archive of oral pathology department to include all data such as onset of symptom and duration of lesions and if there is any associated diseases and the report of radiography.

We recommend making comparison between primary and recurrent cyst by bcl2 marker .

We recommend more investigate the behaivour of OKC by immuno histochemical study to obtain more informations to confirm if this lesion tend to cyst or tumor category especially after the newest classification of WHO in 2017 which reclassified the lesion as a cyst and renamed now odontogienickeratocyst.

Chapter 9

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APPENDICES

THESIS PROPOSAL

Immunohistochemical Study For Bcl-2 in Odontoginic Keratocyst

دراسة مناعية نسيجية كيميائية لتحديد العامل ب س ل – 2 فى الكيس السنى التقرنى

By

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Thesis proposal
Submitted in partial fulfillment of requirement
for the degree of master of science
in oral pathology

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2009

Introduction

The Odontoginic Keratocyst(OKC) Is a relatively uncommon lesion which has much interest because of it[s] unusual growth pattern and tendency to recur [(1)]. Odontoginic Keratocyst occur over a wide age range, but there is pronounced peak incidence in the second and third decades with a second smaller peak in the fifth decade. The cysts are more common in males than females, and 70-80 per cent occur in the mandible. the most common site, accounting for at leas 50 per cent of all cases, in the third molar region and ascending ramus of the mandible. In maxilla the majority of cysts occur in the region posterior to the first premolar [(1,2)].

Odontoginic Keratocyst give rise to remarkably few symptoms, unless they become secondarily inflamed, and this probably accounts for why some do not present until the fifth decade. Contrast to radicular and dentigerous cysts, OKC tend to expand in ananteropo-sterior direction and can reach large sizes without causing gross bony expansion they are often discovered on routine radiographic examination[(3)].The majority of Keratocysts arise sporadically and present as solitary lesions, although in a few patients two or more cysts may develop[(4)]. Multiple cysts are associated with the naevoid basal cell carcinoma syndrome (Gorlin syndrome), inherited as an autosomal dominant trait with variable expressivity, the syndrome is uncommon, but patients with multiple Keratocysts alone may be suffering from it in one of it[s] least expressed forms [(4)].An important clinical feature of Odontoginic Keratocysts is their tendency to recur after surgical treatment. Recurrence rates vary in different reported series from around 3 percent to about 60 per cent [(5)].

It is likely that the rate is decreasing with improved management following recognition of this problem[5]. Radiographically, Keratocysts appear as well-defined radiolucencies that may be unilocular or multilocular many present in apparent dentigerous relationship associated with unerupted third molars but the crowns of such teeth are usually separated from the cyst cavity, the pericoronal tissues being continuous with the cyst capsule. Keratocysts may also present as developmental lateral periodontal cysts[6].There are two types of Odontoginic Keratocyst parakeratotic and orthokeratotic.

The parakeratotic type forms 85 to 95% of all Odontoginic Keratocysts; the balance is made up of the orthokeratinzed variant[6].

Histologic distinction between the para-and orthokerat-inzed variants is made because there is a difference in behavior; the latter is less aggressive, with a much lower rate of recurrence. In the orthokeratotic Odontoginic Keratocyst , a prominent granular layer is found immediately below a flat noncorrugated surface[6]. The basal cell layer is less prominent , with a more flattened or squamous appearance in comparison with the parakeratotic type[6] . The nomenclature and classification of odontogenic tumours are based on those recommended by the World Health Organization (WHO) (1992) and it's Concensus Conference , (2003) . The latter redesignated the Odontogenic Keratocyst as the Keratinizing cystic odontogenic tumour[1].The Bcl-2 proto-oncogene is a member of a gene family that includes cell death suppressors and cell death promoters. Its gene product, the protein Bcl-2,is a 26 kDa putative membrane B associated protein which acts as a cell death suppressor that facilitates cell survival by regulating apoptosis . Investigations on the immunoreactivities of Bcl-2 protein[7,8,9].

have been demonstrated in tooth germs, ameloblastomas, OKCs and dentigerous cysts[7,8,9].

Odontogenic Keratocycts present an aggressive clinical course with a marked trend toward recurrence as compared with dentigerous cysts,differences in the clinical behavior of cysts may be associated with apoptosis in the lining epithelium [10], therefore we make this study to see the expression of apoptosis-related factor Bcl-2 in Odontogenic Keratocyct by immune-histochemical analysis [10].

Aim of Study

To analyze the clinicopathologic features of the selected Odontoginic Keratocyst cases.

To study the immuno -expression of Bcl-2 in Odontoginic Keratocyst.

Methods and Materials

Materials:

- 20 cases of Odontoginic Keratocyst including ortho and para Odontoginic Keratocyst will be collected from the archives of oral pathology department, Faculty of dentistry, Al-Arab medical university Benghazi-Libya.
- The clinical data such as age, sex and site are to be noted from the patient[s] records.
- Antibodies : anti Bcl-2 antibody
- Coated glass slides.
- Kit for Avedin-Bioten-Complex (**ABC**) immunohistochemical studies[11].

Methods:

Hematoxylin and Eosin Staining:

Four microns thick sections will be cut and stained with Hematoxilen and according to (Cullin, Alison and Bar) [12] Sections will be Eosin (H&E) examined to confirm the diagnosis for Odontoginic Keratocyst

Immunohistochemical Staining:

1. Formalin-fixed, paraffin-embedded tissue blocks were sliced at 4 microns thickness and mounted on coated glass-slides.
2. Sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide for antigen retrival
3. They were heated in an autoclave(121c,2atm) in 0.01M citrate buffer (PH 6.0) for 10
   minutes.
4. After treatment with normal serum; the section were incubated with primary antibodies at 4c overnight.
5. The applied antibodies were anti-bcl-2 monoclonal antibody.
6. The standard labeled streptavidin-biotin-peroxidase complex method was preformed to bind the primary antibody by a Histofine SAB-PO kit(Nichirei, Tokyo, Japan)
7. Reaction were visualized by immersing the section in 0.03% diamino-benzidine (DAB) solution containing 2mM hydrogen peroxide for 3 to 5 min.
8. The immunostaining reaction of survivin and bcl-2 were evaluated according to intensity and patterns of distribution,
9. Intensity of staining was graded as weak, moderate and strong.
10. The section were then counterstained with mayer,s hematoxyline and examined by light microscope (kichi etal) [11].

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Questionnare

Case 1 (biopsy no)

1 **Clinical data :-**

a **age** ……..

b **sex** ……..

c **site** ……..

d **other finding** ……..

2 **Histopathological features :-**

A **Cyst lining**

1 **Stratified squamous epithelium**

thick ☐ **thin** ☐

2 **Basal layer**

palisaded ☐ **not palisaded** ☐

3 **Surface layer**

parakeratinized ☐ **or thokeratinized** ☐

4 **Mitotic figure**

few ☐ **nemours** ☐

Basal ☐ **supra basal** ☐

B Cyst wall

1 Inflammatory cells

present ☐ → acute ☐
present ☐ → chronic ☐
absent ☐

2 Satellite cyst

present ☐ absent ☐

3 Epithelial residues

present ☐ absent ☐

C Cyct cavity

Kerattine squames

present ☐ absent ☐

3 Bcl2 expression :-

a Positive ☐ Negative ☐

b Mild ☐ Moderate ☐ intense ☐

c Diffuse ☐ → basal ☐
Diffuse ☐ → Supra basal ☐
Diffuse ☐ → Others ☐

E Localized ☐ → Lasal ☐
Localized ☐ → Supra basa ☐
Localized ☐ → Others ☐

D Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 91:05)

1 **Clinical data :-**

a age 35 years

b sex male

c site mandible

d other finding /

2 **Histopathological features :-**

A **Cyst lining**

1 **Stratified squamous epithelium**

thick ☐ thin ☑

2 **Basal layer**

palisaded ☐ not palisaded ☑

3 **Surface layer**

parakeratinized ☑ or thokeratinized ☐

4 **Mitotic figure**

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐

present ☑ → chronic ☑

absent ☐

2 Satellite cyst

present ☑ absent ☐

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☑ intense ☐

c Diffuse ☑ → basal ☑

Supra basal ☑

Others ☐

Localized → basal ☐

Supra basal ☐

Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 55-01)

1 **Clinical data :-**

a age 23 years

b sex male

c site Rt side of mandible.

d other finding ✓

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
present ☑ → chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☑ intense ☐

c Diffuse ☑ → basal ☑
Supra basal ☑
Others ☐

Localized ☐ → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 90.03)

1 Clinical data :-

(a) age 35 years

(b) sex male

(c) site mandible

(d) other finding /

2 Histopathological features :-

A Cyst lining

(1) Stratified squamous epithelium

thick ☑ thin ☐

(2) Basal layer

palisaded ☐ not palisaded ☑

(3) Surface layer

parakeratinized ☑ or thokeratinized ☐

(4) Mitotic figure

few ☐ nemours ☑

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑

absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☐ intense ☑

c Diffuse ☑ → basal ☑
Supra basal ☑
Others ☑

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 156-98)

1 **Clinical data :-**

a age ...24 years.....

b sex .....male.....

c site ...Rvam ~~14-6~~ region -

d other finding .....

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☑ thin ☐

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☐ nemours ☑

Basal ☑ supra basal ☑

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyst cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☐ Negative ☑

b Mild ☐ Moderate ☐ intense ☐

c Diffuse → basal ☐
Supra basal ☐
Others ☐

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 84.011)

1 **Clinical data :-**

a age ..65 years...

b sex ...male.....

c site ..Rt. cheak.....

d other finding ...../.....

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☐ → acute ☐
present ☐ → chronic ☐
absent ☐

2 Satellite cyst

present ☑ absent ☐

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☑ intense ☐

c Diffuse ☑ → basal ☑
Diffuse ☑ → Supra basal ☑
Diffuse ☑ → Others ☐

Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

d Nuclear ☑ Cytoplasmic ☑ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 44.07)

1 Clinical data :-

a age 27 years

b sex male

c site from 13-6 region

d other finding /

2 Histopathological features :-

A Cyst lining

1. Stratified squamous epithelium

   thick ☐ thin ☑

2. Basal layer

   palisaded ☐ not palisaded ☑

3. Surface layer

   parakeratinized ☑ or thokeratinized ☐

4. Mitotic figure

   few ☑ nemours ☐

   Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑
absent ☐

2 Satellite cyst

present ☑ absent ☐

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☐ intense ☑

c Diffuse ☑ → basal ☑
Supra basal ☑
Others ☑

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 72:09)

1 Clinical data :-

a age 42 years

b sex male

c site Rt side of mandible

d other finding /

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☑ thin ☐

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☐ intense ☑

c Diffuse ☑ → basal ☑
Supra basal ☑
Others ☑

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 3.a.14)

1 **Clinical data :-**

a age 21 years

b sex female

c site mandible

d other finding /

2 **Histopathological features :-**

A **Cyst lining**

1 **Stratified squamous epithelium**

thick ☐ thin ☑

2 **Basal layer**

palisaded ☑ not palisaded ☐

3 **Surface layer**

parakeratinized ☐ or thokeratinized ☑

4 **Mitotic figure**

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑

absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☑ intense ☐

c Diffuse ☑ → basal ☑
Supra basal ☑
Others ☐

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☑ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 22.011)

1 **Clinical data :-**

a age 40 years

b sex female

c site ~~lt~~ lt region.

d other finding .....

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☐ or thokeratinized ☑

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

① Inflammatory cells

present ☐ → acute ☐
present ☐ → chronic ☐
absent ☑

② Satellite cyst

present ☐ absent ☑

③ Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

③ Bcl2 expression :-

ⓐ Positive ☑ Negative ☐

ⓑ Mild ☑ Moderate ☐ intense ☐

ⓒ Diffuse ☑ → basal ☑
Diffuse ☑ → Supra basal ☑
Diffuse ☑ → Others ☐
Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

ⓓ Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 27.03)

1 **Clinical data :-**

ⓐ age 25 years

ⓑ sex female

ⓒ site mandible

ⓓ other finding /

2 **Histopathological features :-**

A **Cyst lining**

① Stratified squamous epithelium

thick ☐ thin ☑

② Basal layer

palisaded ☑ not palisaded ☐

③ Surface layer

parakeratinized ☑ or thokeratinized ☐

④ Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
present ☑ → chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☐ Negative ☑

b Mild ☐ Moderate ☐ intense ☐

c Diffuse → basal ☐
Diffuse → Supra basal ☐
Diffuse → Others ☐

Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

d Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 10-014)
10-014

1 **Clinical data :-**

a age 32 Years

b sex Female

c site Lower Rt side of mandible

d other finding .....

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☐ or thokeratinized ☑

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☐ → acute ☐
present ☐ → chronic ☐
absent ☑

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☐ Negative ☑

b Mild ☐ Moderate ☐ intense ☐

c Diffuse → basal ☐
Diffuse → Supra basal ☐
Diffuse → Others ☐
Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

d Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 125.98)

1 **Clinical data :-**

a age 20 years

b sex male

c site Rt. ascending ramus of the mandible.

d other finding /

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☐ Negative ☑

b Mild ☐ Moderate ☐ intense ☐

c Diffuse → basal ☐
Supra basal ☐
Others ☐

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 61.013)

1 **Clinical data :-**

a age 27 years

b sex male

c site in the L-S region

d other finding /

2 **Histopathological features :-**

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☐ → acute ☐
present ☐ → chronic ☐
absent ☑

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☑ Moderate ☐ intense ☐

c Diffuse ☑ → basal ☑
Diffuse ☑ → Supra basal ☐
Diffuse ☑ → Others ☐

Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 58-97)

1 Clinical data :-

a age ..64 Years..

b sex ..male..

c site ..Rt. Ramus of mandible..

d other finding ..../..

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☐ or thokeratinized ☑

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐ / chronic ☑

absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☐ Negative ☑

b Mild ☐ Moderate ☐ intense ☐

c Diffuse → basal ☐ / Supra basal ☐ / Others ☐

Localized → basal ☐ / Supra basal ☐ / Others ☐

d Nuclear ☐ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 120.06

1 Clinical data :-

a age 27 years

b sex male

c site maxilla from 6-11 region

d other finding ✓

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☑

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
present ☑ → chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☑ intense ☐

c Diffuse ☑ → basal ☑
Diffuse ☑ → Supra basal ☐
Diffuse ☑ → Others ☐
Localized → basal ☐
Localized → Supra basal ☐
Localized → Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 128,01)

1 Clinical data :-

a age 48Years

b sex female

c site in the palate related to 2-1 region

d other finding /

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☑ thin ☐

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☐ absent ☑

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☑ Moderate ☐ intense ☐

c Diffuse ☑ → basal ☑
Supra basal ☐
Others ☐

Localized → basal ☐
Supra basal ☐
Others ☐

d Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

219.97

Case 1 (biopsy no)

1 Clinical data :-

a age ...44...years...

b sex .....male.....

c site .....from ~~to~~ 12.34 region.....

d other finding ...../.....

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☑ → acute ☐
present ☑ → chronic ☑
absent ☐

2 Satellite cyst

present ☐ absent ☐

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☐ intense ☑

c Diffuse ☑ → basal ☑
Diffuse ☑ → Supra basal ☑
Diffuse ☑ → Others ☐

E Localized ☐ → Lasal ☐
Localized ☐ → Supra basa ☐
Localized ☐ → Others ☐

D Nuclear ☑ Cytoplasmic ☑ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 100.97)

1 Clinical data :-

a age ...18 years.....

b sex .....Female.....

c site .....impacted 3/.....

d other finding ...../.....

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☐ not palisaded ☑

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☐ nemours ☑

Basal ☑ supra basal ☐

B Cyst wall

① Inflammatory cells — present ☑ → acute ☐ / chronic ☑ ; absent ☐

② Satellite cyst

present ☑ absent ☐

③ Epithelial residues

present ☐ absent ☑

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

ⓐ Positive ☑ Negative ☐

ⓑ Mild ☐ Moderate ☐ intense ☑

ⓒ Diffuse ☑ → basal ☑ / Supra basal ☑ / Others ☐

Ⓔ Localized ☐ → Lasal ☐ / Supra basa ☐ / Others ☐

Ⓓ Nuclear ☑ Cytoplasmic ☑ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 61.89)

1 Clinical data :-

a age 28 Years

b sex male

c site mandible

d other finding /

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☑ or thokeratinized ☐

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

1 Inflammatory cells

present ☐ → acute ☐

chronic ☐

absent ☑

2 Satellite cyst

present ☐ absent ☑

3 Epithelial residues

present ☑ absent ☐

C Cyct cavity

Kerattine squames

present ☑ absent ☐

3 Bcl2 expression :-

a Positive ☑ Negative ☐

b Mild ☐ Moderate ☐ intense ☑

c Diffuse ☑ → basal ☑

Supra basal ☑

Others ☐

E Localized ☐ → Lasal ☐

Supra basa ☐

Others ☐

D Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Case 1 (biopsy no 94-04)

1 Clinical data :-

a age ....20 Years....

b sex ....Female....

c site ....Rt. maxilla....

d other finding ....../.....

2 Histopathological features :-

A Cyst lining

1 Stratified squamous epithelium

thick ☐ thin ☑

2 Basal layer

palisaded ☑ not palisaded ☐

3 Surface layer

parakeratinized ☐ or thokeratinized ☑

4 Mitotic figure

few ☑ nemours ☐

Basal ☑ supra basal ☐

B Cyst wall

① Inflammatory cells — present ☑ → acute ☐ / chronic ☐ ; absent ☐

② Satellite cyst — present ☐ absent ☑

③ Epithelial residues — present ☐ absent ☑

C Cyct cavity

Kerattine squames — present ☐ absent ☑

3 Bcl2 expression :-

ⓐ Positive ☑ Negative ☐

ⓑ Mild ☐ Moderate ☐ intense ☑

ⓒ Diffuse ☑ → basal ☑ / Supra basal ☑ / Others ☐

Ⓔ Localized ☐ → Lasal ☐ / Supra basa ☐ / Others ☐

Ⓓ Nuclear ☑ Cytoplasmic ☐ Cell membra_nous ☐ Extra cellula ☐

Abstract Arabic

دراسة مناعية نسيجية كيميائية لتحديد العامل ب س ل -2

في الكيس السني التقرني

قدمت من قبل :

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الملخص

المقدمة :- الكيس السني التقرني نادر الحدوث نسبيا ولكن سلوكه جدير بالاهتمام والدراسة لأن لديه نمط نمو غير تقليدي ومختلف عن باقي الأكياس السنية وميول كبير للحدوث مجددا بعد إزالته جراحيا.

معظم حالات الكيس التقرني تحدث بدون سبب وعادة ما يكون كيس واحد إلا إذا كان مصحوبا مع متلازمة ورم الخلايا القاعدية الخبيثة . الكيس السني التقرني ممكن أن يحدث في أي مكان في الفكين ,لكن المكان الأكثر شيوعا لحدوث هذا الكيس هو الفك السفلي وخاصة الجزء الخلفي من جسم الفك السفلي .

الكيس السني التقرني يظهر سلوك سريري عنيف ولديه ميول كبير للظهور مجددا مقارنة بالأكياس السنية الأخرى . الاختلاف في السلوك للكيس يكون معتمدا على موت الخلايا في البطانة الطلائية .

الهدف من الدراسة :1 -لتحليل المظاهر النسيجية المرضية لحالات الكيس السني التقرني .

2- دراسة وصفية مناعية للعامل BCl2 (ب س ل . 2) في الكيس السني التقرني .

المواد و الطريقة :-دراسة وصفية لعينات الكيس التقرني عددها 20 حالة مشخصة مسبقا أنها كيس سني تقرني

البيانات والمعلومات جمعت من أرشيف قسم أمراض الفم في كلية الأسنان جامعة بنغازي في الفترة الممتدة من يناير 1997 إلى ديسمبر 2014 .البيانات الشخصية للحالات مثل العمر – الجنس – العنوان – الجنسية – ومكان حدوث الكيس جمعت من الأرشيف .أخذت العينات من القوالب الشمعية وقطعت وصبغت و سمك القطاع كان 4 ميكرون وصبغ بصبغة هيموتوكسلين مع الايوسين (H & E) ومن ثم تم صبغه العامل BCl2 في قسم أمراض الفم بجامعة الإسكندرية لدراسة التفاعل المناعي للعامل BCl2 في الخلايا الطلائية للكيس .أخيرا جمعت جميع المعلومات وحللت بطريقة احصائية ببرنامج SPSS الاصدار 17

النتائج :–عمر الحالات كان يتراوح من 18 – 65 سنة المتوسط العمري = 33.25 سنة

ونسبة حدوث الكيس ذكر : انثى 1.9 : 1 معظم الحالات حدثت في العقد الثاني إلى العقد الثالث من العمر .

كل الحالات كانت ليبية الجنسية وجميعها من مدينة بنغازي فيما عدا حالة واحدة من مدينة طبرق .

معظم الحالات كان الكيس في الفك السفلي وكل الحالات حدثت على هيئة كيس واحد ولايوجد أي حالة متعددة الأكياس .الوصف النسيجي المرضي للكيس السني التقرني يتميز أولا بنسيج طلائي حرشفي مركب رقيق في معظم الحالات . ويتميز النسيج بخلايا قاعدية مطوقة وسطح الكيس مبطن بطبقة تقرنية .

جدار الكيس يحتوي على :خلايا التهابية في النسيج الضام في 75% من الحالات وأكياس تابعية موجودة في 40% من الحالات و بقايا طلائية في 40% من الحالات و أكوام تقرنية تملأ الكيس في أكثر من نصف الحالات

بالنسبة للخلايا التي تفاعلت مع صبغة العامل Bcl2 كانت ايجابية النتيجة ونسبة التفاعل عالية والتفاعل كان قوي في 75% من الحالات وكان منتشر وليس موضعي وصنف التفاعل إلى ثلاثة أنواع – خفيف – متوسط – قوي . بنسبة 20% – 33.3% – 46.6% على التوالى .فتوزيع التفاعل الايجابي في الخلايا

القاعدية و فوق القاعدية موجود في معظم الحالات وله أهمية قصوى ولهذا فان التفاعل المناعي موجود في الخلايا القاعدية وفوق القاعدية .–خلصنا في نهاية هذه الدراسة الي أن حالات الكيس التقرني السني المدروسة كانت وحيدة غير متعددة – لا يوجد حدوث متكرر لها . معظم الحالات له سلوك عنيف لذلك كان يجب علينا تصنيفها إلى ورم بدل من كيس بسبب أن معظم الحالات لديها قدرة انقسامية عالية جدا لذلك تغيير المرض نموي مع سلوك سرطاني الخصائص والنتائج المناعية الكيميائية أثبتت وجود تفاعل إيجابي للعامل Bcl2 في 75% من الحالات

في نهاية الدراسة نوصى الاطباء بضرورة كتابة الملفات طبية بصورة كاملة حتى تصف الحالة الصحية للمرضى وتوفير معلومات أكثر عن بداية الأعراض ومدة المرض والأمراض الأخرى المصاحبة وتقارير الأشعة التى يجب ان تكون موجودة في أرشيف القسم وكذالك نوصي بالمزيد من الابحاث للكيس السنى التقرنى لدراسة توزيع الخلايا ايجابية التفاعل للعامل ب س ل –2 عن طريق تقييم الاكياس الاولية .

دراسة مناعية نسيجية كيميائية لتحديد العامل ب س ل -2 في الكيس السني التقرني

قدمت من قبل:

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جامعة بنغازي

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