Study the effect of age on Histochemical, Histometrical and Immune Histological finding of Submandibular gland in buffalo

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ABSTRACT

In this study, twenty health heads of buffalo in both sex were used and collected from Basrah slaughter house, then divided into two groups according to the age of the animal (one years old and five years old) ,each age involved ten heads of the animals. The general histological finding of submandibular gland was showed a fibrous connective tissue capsule from which septa extend and divided the gland into lobes and lobules. Tubuloacinar- type was the characteristics of the gland. The acini were serous and mucous. The secretory ducts were consisting from : intercalated, striated , inter lobular and inter lobar ducts. The myoepithlial cells which located around the acni and intercalated ducts. The age was effected on the connective tissue and fibrosis were more intensity with gland at five years old of animal, while the glycogen was more intensity with gland at one year old in comparison with other age. The immunhischemistry study was showing a high distribution of brown to gray color of VEGF (vascular endothelial growth factor) around the acni, ducts and blood vessels in the one year old in compare with the five years old (low distribution). The statistical analysis of histometric study showed a significant differences at $p<0.05$, the mean diameter of serous acni, intercalated ducts, striated ducts and interlobular ducts were increased at five years old (14.95±5.34, 13.13±3.23, 26.27±1.33 and 64.68±7.43) µm respectively in compare with one years old of animals (8.32±2.88, 6.12±0.98, 21.11±1.83 and 54.81±1.96) µm.

KEYWORDS: Submandibular salivary gland, buffalo, Age

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INTRODUCTION

The oral cavity is underlined by the mucosal membrane and always moistened by the secretion of associated glands, that which divided into two parts, the first part was named the major salivary glands and the other was named the minor salivary glands [1]. In mammals, The major ones are the parotid, mandibular and sublingual glands while, the minor salivary glands are named according to their location, such as labial, buccal, molar, palatine, lingual and paracaruncular glands [2]. Salivary glands play important roles in the digestive system, especially in ruminants. The main secretions were divided in two parts (serous and mucous), containing many enzymes, carbohydrate, water, mucopolysaccharides (complex carbohydrates) and glycoprotein that that initiated the digestion, moistened, lubricant and digestion of the food . However, the saliva of ruminants contains no digestive enzymes [3,4] .The present study was aimed to study the histochemical, histometrical and immunhistological of submandibular gland in buffalo according to the age.

MATERIALS & METHODS

Twenty healthy heads of buffalo on both sex with ages (at one year and five years old) from the Basra slaughter house were used in this study . The submandibular glands were collected directly and removed from the adjacent structures, then followed by a serial steps and processes according to the [5]. Then, the histological section was stained with Heamatoxyllin and eosin stain for study general histological structures, PAS reaction for detection the glycogen in the submandibular gland, and some of these sections were used in the immunohistochemical study with the process, vascular endothelial

growth factor(VEGF). The sections were examined and photographed by using light microscope and statically evaluated by using a T-test program [6].

RESULTS

The mandibular gland was lobulated gland surrounded by a fibrous, dense connective tissue capsule (collagenous, elastic and few reticular fibers) from which septa was extended and divided the gland into lobes , each lobe was subdivided into lobules, the gland was tubloacinar in type , the secretory system of the gland was involved acini (serous, mucous and serous Demilune) and the ducts (Figure.1.A and B) and (Figure3). In both age the serous acini were small in size and diameter of the lumen, dark rounded in shape and lined by pyramidal cells with a round nucleus which located at the base of the cell , while the mucous acini was larger and more variable in size and shape with the lightening color and lining by cuboidal shaped cells that had flattened nucleus (Figure2). The secretory ducts, composed intercalated ducts that small in size with the cuboidal cell lining, striated duct, which large in size with columnar epithelium lining , interlobular ducts that present within the lobules lined by simple columnar epithelium and intralobular ducts that located within the septa with the psedustratified cuboidal epithelium (Figure 1 and Figure 2 A). The myoepithelial cell was present around the acni and intercalated duct(Figure3). The connective tissue was observed more clearly in the submandibular gland at five years of age of the animals in comparing with the other group at one year ago, which contain less intensity of connective tissue in the septa and around the acini (Figure3). The fibrosis were prominent in the mandibular gland at five years old of the animals in comparison with one year old of animals (Figure3). In histochemestry observation the PAS stain was used to demonstration the Glycogen in the tissues that was purple in color and more intensity in the tissue of mandibular gland at one year old of animals in compare with the other groups which appeared lesser (Figure 4).The immunhistochmistry of the mandibular gland was observed by using VEGF that appeared as brown to gray in color around the acini , ducts and blood vessels which distributed more obviously in gland with the one year of animals age in compare to the five years of the age which appeared in less amount (Figure5). The statistical analysis show there was significant differences P<0.05 in the mean diameter of serous acni that increased in the five years old of age (14.95±5.34) µm in compare with one years (8.32±2.88) (Table.1), also there were significant increase P>0.05 in the mean diameter of secretory ducts (intercalated duct, striated duct, interlobular duct) in five years old (13.13±3.23, 26.27±1.33and 64.68±7.43)µm respectivelyin compare with mean diameter of one years old of animal (6.12±0.98, 21.11±1.83 and 54.81±1.96)µm (Table.2)

Table (1): Mean diameter of mandibular acni (serous and mucous) in buffalo(µm)

Mandibular acni	Serous acni	Mucous acni
One years	**8.32±2.88**	**21.32±2.53**
Five years	**14.95±5.34***	**24.89±4.81**

***= significant differences P<0.05.**

Table (2): Mean diameter of mandibular ducts in buffalo (µm)

Mandibular ducts	Intercalated duct	Striated duct	Interlobular duct
One years	**6.12±0.98**	**21.11±1.83**	**54.81±1.96**
Five years	**13.13±3.23***	**26.27±1.33***	**64.68±7.43***

***= significant differences P<0.05.**

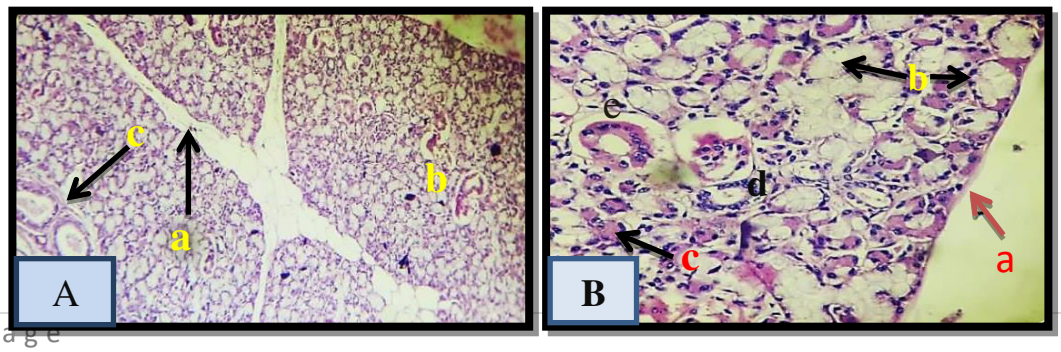

Figure.1:Histological section of submandibular gland of buffalo show: A: a.Septa, b.lobule, c. interlobular duct. H&E stain 10X B: a. Capsule, b. Mucous acni, c. Serous acni, d.intercalated, e. striated duct. H&E stain 20X

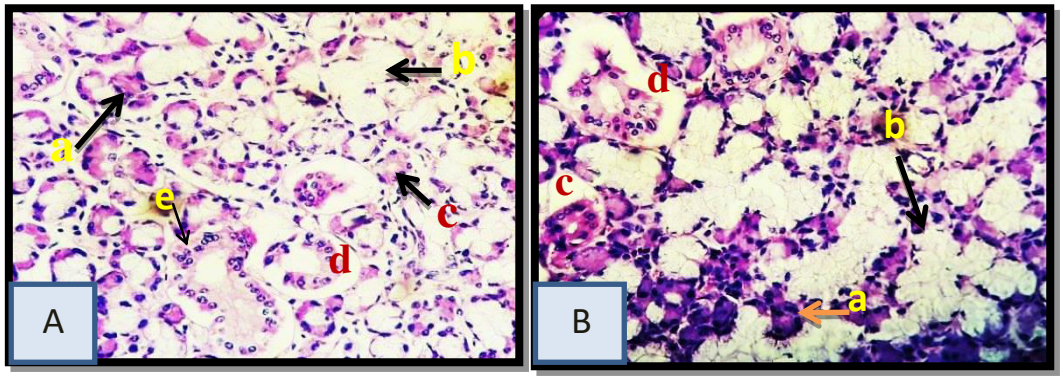

Figure.2 Histological section of mandibular gland show A. at one year show normal diameterin in a. serous acni, b. mucous acni, c.intercalated duct, d.straited duct e. interlobular duct B: at five years show increase in diameter of aserousacni, b.mucousacni, c.intercalated duct , d.straited duct. H&E stain. H&E.20X

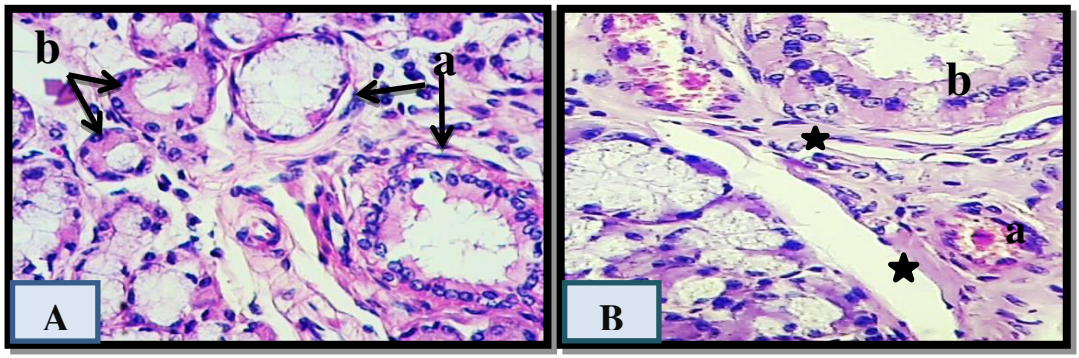

Figure.3: Histological section of submandibular gland show A: At one years old a. Myoepithelial cell, b. Serous demilune, normal intensity of connective tissues, no fibrosis B: At five years old: High intensity of connective tissue in the septa with few fibrosis (★), a. Blood vessels, b. Interlobar duct. H&E. 40X

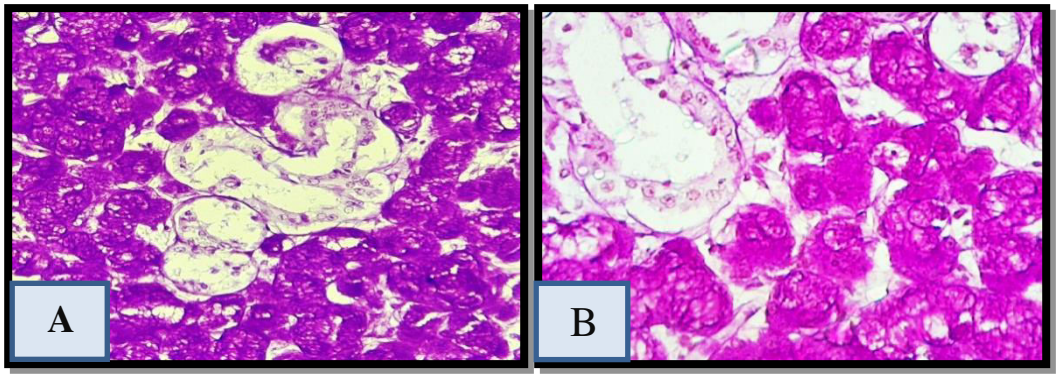

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Figure.4:Cross section of submandibular gland show: A: High intensity of glycogen (deep purple) at one years old (B) At five years there was low intensity of glycogen (light purple).PAS stain 40X

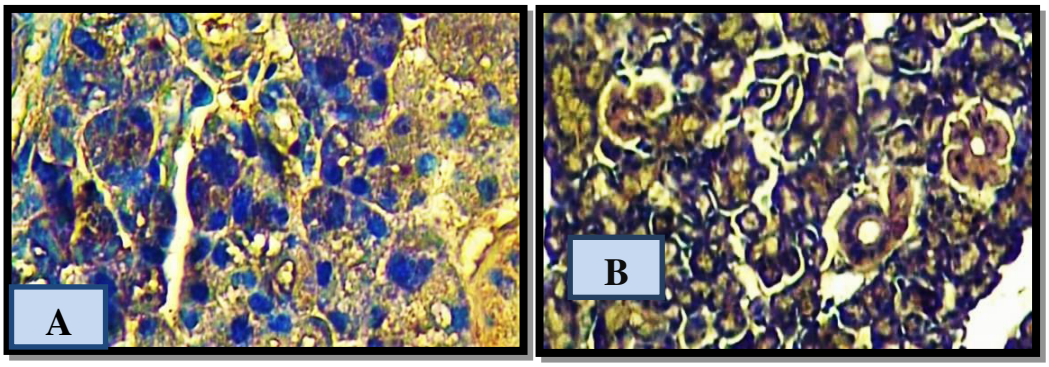

Figure.5: Immuonhistochemistry of sub mandibular salivary gland show: (A) Low deposition of VEGF (brown color) at five years old, (B) At one year old high localization of VEGF (dark brown color) in the center of acni and ducts. 20X

DISCUSSION

The mandibular gland was encapsulated by fibrous connective tissues from this capsule the septa was extended to form the lobes and lobules, this was this a similar feature has been reported in bovine [7], in camel [8] in horse [9,10] in goat. The secretion of mandibular gland was mixed in appearance, therefore the histological section was composed of two types of secretory unit serous and mucous acni, this was agreed with [11,12] in bovine and with [13] in barking deer (*Muntiacusmuntjak)* and not agree with the cat and dog according to the [12]. The demilunes of the mandibular gland was different with species of animals, in buffalo was reported seromucous and this coincides with [14] in calf, [15] in sheep and [16] in camel. The secretory ducts of the gland divided into many parts, intercalate, striated, interlobular and excretory duct, the intercalated duct was lined by simple cuboidal cell and this similar to the results which reported in [17] in camel,ox, sheep and goat. The striated duct was lined by simple columnar epithelium, this result was agreed with [10] in goat and [8] in camel. The interlobular duct was lined by simple columnar cells and the interlobar duct was lined by stratified columnar cell as in camel [7,16] in bovine and [10] in goat . It was demanded that the presence of stratified epithelium in the interlobular duct may reflect the need for protection of underlying basement membrane for occasional action of activated serous fluid enzymes [18]. In the present study the gland showed a PAS-positive reaction which indicated the increased the glycogen in the sub mandibular gland at one year old in comparing with five years old, this were in agreement with [16] in camel, [15] in mammals , [19] in rabbits and [20] in mice who mentioned that the fibrotic regions were rich in collagens and it has been investigated that the total collagen density was lower in young mice than in older mice. In addition, acinar atrophy and dilated interlobular ducts were observed in old mice. The myoepithelial cells were present between the base of the acinar cells and the basal lamina in both ages this similar to the result which reported by of [21] in rabbits and cats while, disagree with [22] in rat who reported that, the serous acini lack myoepithelial cells.

The intensity of connective tissues were increased in the submandibular salivary glands with age of five years as compared to the gland with age of one year this, agree with [23-26] in human; [27,28] in rats ,who said the major salivary gland tissue represented by acinar cells with age, serous and mucous acinar cells are lost or decrease in the number and replaced by fat, connective tissue due to aging. The increase in adipose tissue by aging may be attributed to the reduction in the hormone Testosterone which associated with aging [29] in human; [26] in rats .

The fibrosis was present clearly in five years old in comparison with one year old ,this was coincided with [30] whose reported there were higher levels of periductal fibrosis in the salivary gland in adult rats, [31] in human and [32] in rat.

The VEGF was more clearly in mandibular gland with one year ago in comparing with the five years old of animals that appear in the section as brown to gray color around the blood vessels and the base of acini and ducts, this was confirmed by [33,34,35] whose reported the vascular factors was important roles in the formation of newly blood vessels during the embryonic life and dose not persist during the life.

The mean diameter of serous acni was increased gradually with developments of the age , it was increased significantly P<0.05 (14.95±5.34)in five years old as a compared with mean of one years old which was (8.32±2.88) , this was an agreement with [36,37]. The mean of intercalated duct , striated duct and interlobular duct was increased significantly P>0.05 with developing of the age in five years these ducts was in high value ((13.13±3.23, 26.27±1.33and 64.68±7.43)µm, more than in one year old (6.12±0.98, 21.11±1.83 and 54.81±1.96)µm, this was agreed with [38] human and [39] in rats.

CONCLUSION

This study was concluded the histochemical, histometrical and immune histological finding of submandibular gland was affected with age of animals, the connective tissue and fibrosis were appeared high intensity with animals at five years old. While, the VEGF was more clearly with the one year old of the animals. The mean diameters of serous acini, intercalated ducts,striated ducts and inter lobular ducts of submandibular gland was significantly increased at p<0.05 with the animals at five years old in compare with one year of the age.

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