

# Histological, Histochemical And Immune Histochemical Study of Effect Aging on the Parotid Salivary Glands in Buffalo

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## Abstract

The present work were carried out on twenty parotid salivary glands were collected from head of buffalo (slaughtering of Basra) and divided into two equal groups (one year age and five year age). The results were showed that the parotid salivary gland consist of spherical serous acini connected with branched ducts, intercalated ducts which attached with striated ducts, these duct involved the intra lobular ducts. The larger duct named interlobular duct, myoepithelial cells located between the basal membrane and secretory acini and ducts. The results revealed that the effect of aging on the histological structures of parotid salivary gland by using routine stain, histochemical stain stereological histologically, and immunohistochemistry. The high intensity of connective tissues in the parotid salivary glands with age of five years as compared to the age of one years (normal intensity), also showed fibrosis in the parotid gland of five year age as compared with parotid gland of one year no appear any fibrosis, stereological histologically of the mean diameter of acini for parotid gland of five years age increased significantly  $p < 0.05$  ( $13 \pm 0.33$  micron) while acini of one years age ( $10.4 \pm 0.02$  micron), the mean diameter of intercalated duct for parotid gland of five years age increased significantly  $p < 0.05$  ( $40.13 \pm 2.61$  micron) as compared of intercalated duct for age one years ( $26.56 \pm 1.22$  micron). The histochemical results by using PAS stain showed low intensity of glycogen for parotid gland of five years age as compared to the group of one years age. The immunohistochemistry (VEGF) showed decrease in the parotid salivary gland of five year age as compared to the group of one years age.

**Keywords:** salivary glands, aging, hypo salivation.

## Introduction

The salivary apparatus consist of three major salivary gland and several minor salivary glands<sup>1</sup>. The parotid glands were the one and largest of the three major salivary glands located at the side of maxilla opposite to ear and resemble to pancreas<sup>2</sup>. The parotid salivary glands divided in to lobules by connective tissues, each lobules contain several secretory serous acini, these acini attached to striated ducts which led to inter lobular duct. The parotid salivary gland secret serous saliva which act as protection, antimicrobial, mineralization, lubrication, epithelial repair and buffering<sup>3</sup>. The aging is term used in human, animals, plants and fungi caused by many factor, the first is free radicals which attack the cells and damage the DNA, the second is the imuno system depression which promote the cells and tissues for attack. The third cause is the glycation and oxidative stress<sup>4</sup>. The glycogen consider as the main storage unit

of glucose in the tissues and play an important role in the salivary glands<sup>5</sup> in rat. The aim of this study is to investigate the effect of aging on the histology, some histochemistry and immunohistochemistry of the parotid salivary glands.

## Materials and Method

Twenty parotid salivary glands were collected from buffalo (slaughtering of basrah) and divided into two equal groups. Group I with one year age and Group II with five year age. The deparaffinized sections were stained with hematoxyline eosin for routin histological study and PAS reaction for glycogen content<sup>6</sup>, as well as some deparaffinized section were processed for VEGF immunohistochemistry. The mounted section were photographed with light microscope evaluated statistically using t-test program<sup>7</sup>.

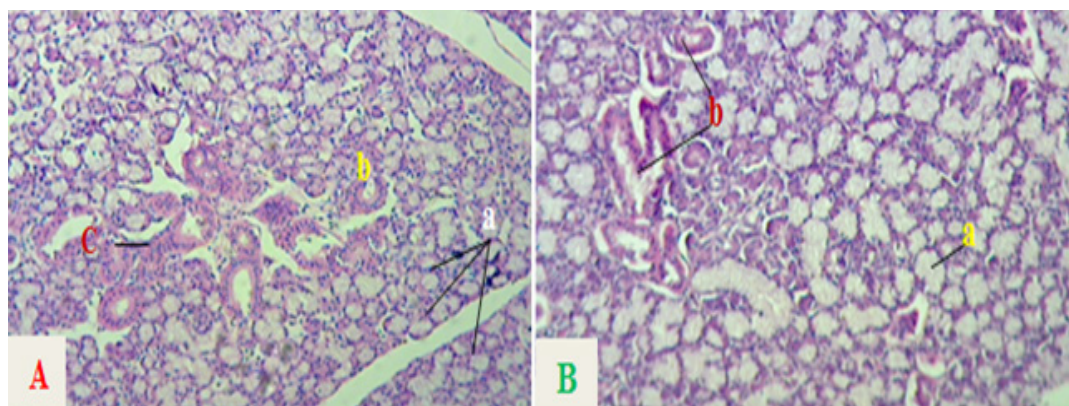
**Results**

The present study showed that parotid salivary gland consist of spherical serous acini connected with branched ducts. There were intercalated ducts which attached with striated ducts , these duct involved the intra lobular ducts .There were larger duct named interlobular duct(Fig.1). There are two type of secretory cells (mucous cells and serous cells, the secretory granules were located in the upper part of cell. There were myo epithelial cells located between the basal membrane and secretory acini and ducts, there were connective tissue dividing the glands to lobes and lobules by trabeculli which contain nerves and blood vessels(Fig.2(B)).When comparing the two group ,the intensity of connective tissues were increased in the parotid salivary glands with age of five years as compared to the parotid salivary gland with age of one years (normal intensity)(Fig.2) .There were a few fibrosis in the parotid salivary gland of five year age as compared with parotid salivary gland of one year which showed no any fibrosis(Fig.3).

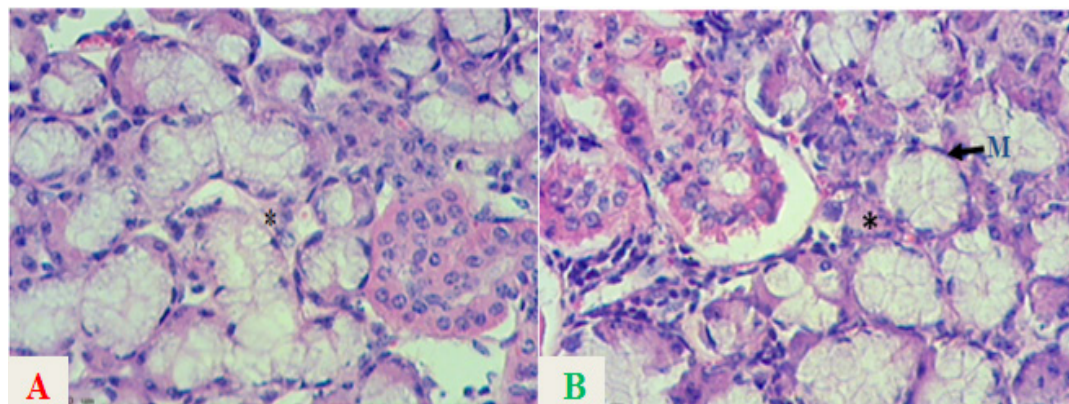
The steriological histologically of the mean diameter of acini for parotid gland of five years age observed increased significantly  $p<0.05$  ( $13\pm0.33$  micron) as compared to the parotid of one years age( $10.4\pm0.02$  micron). The steriological histologically of the mean diameter of intercalated duct for parotid gland of five years age observed increased significantly  $p<0.05$  ( $40.13\pm2.61$  micron) as compared to the parotid of one years age( $26.56\pm1.22$  micron) (Fig.1).

The intensity of PAS reaction(glycogen content) were decreased in the parotid salivary glands of five years age as compared to the group of one years age(Fig.4).

The VEGF immuonohistochemistry showed that VEGF distributed in the basal membrane around the acini, ducts and around the blood vessels in connective tissues in the parotid salivary glands. The results showed that the intensity of VEGF which showed as brown color were decreased in the parotid salivary gland of five year age as compared to the group of one years age (Fig.5).



**Fig.1.**Cross section of parotid gland (A) Five year age showed normal diameter of acini(serous acini)(a) ,(b)intercalated ducts and (C) striated ducts. (B)One year age showed dilated acini(a) and intercalated ducts(b).H.E stain(X40). (B) of one year age showed dilated acini(A) and intercalated ducts(B) .H.E stain(X40)



**Fig.2.**Cross section of parotid gland (A)One year age showed normal intensity of connective tissue(\*).(B) Five year age showed high intensity of connective tissue(\*) ,Myoepithelial cell(M).H.E stain(X40).

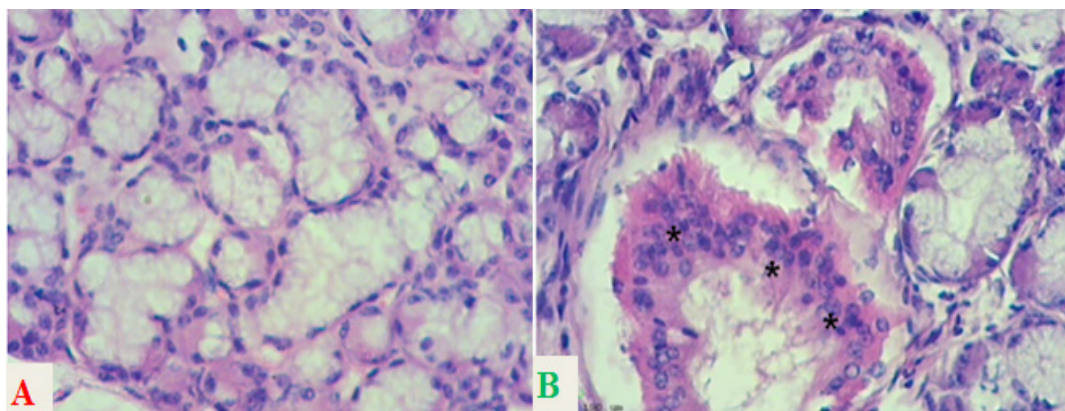


Fig.3.cross section of parotid gland )A)One year age showed no fibrosis(B)Five year age showed fibrosis(\*).H.E stain(X40).

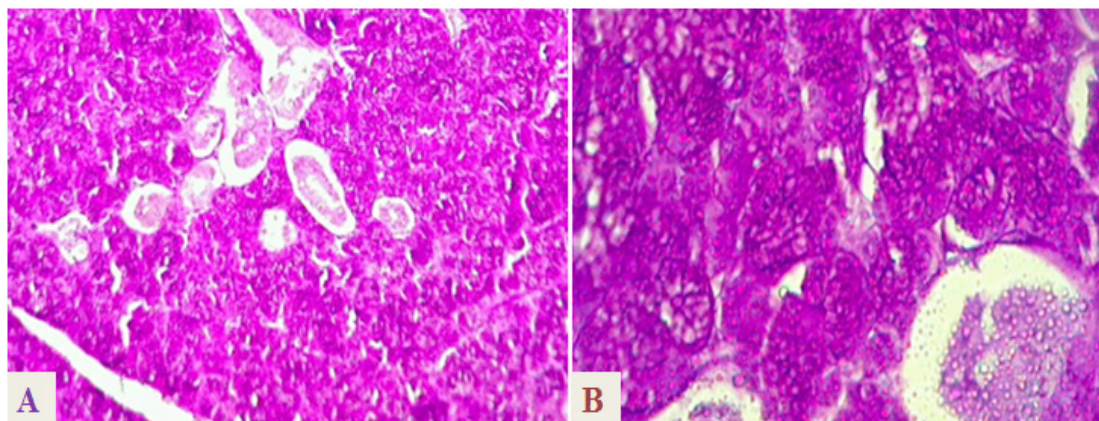


Fig.4.Cross section of parotid gland (A)Five year age showed low intensity of glycogen (purple color)(B)One year age showed high intensity of glycogen(purple color).PAS stain(X40).

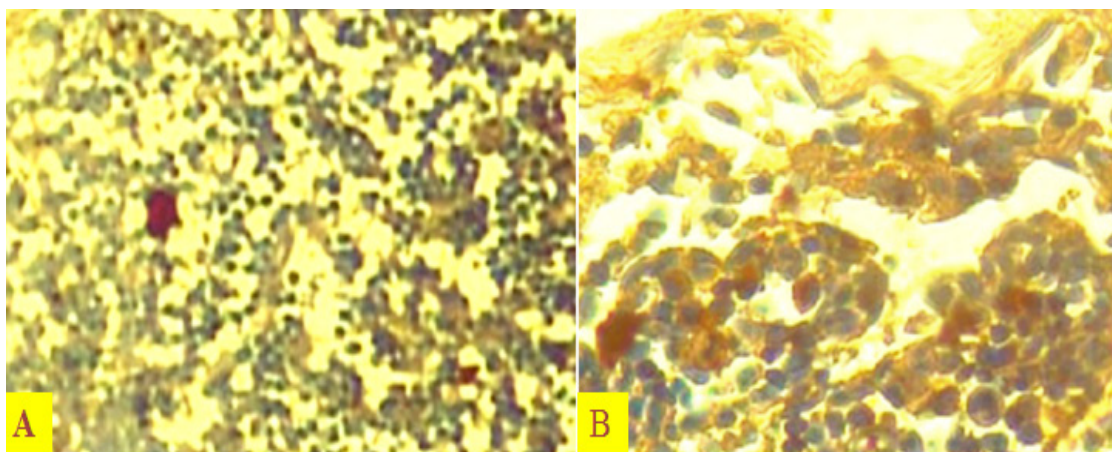


Fig.5.Cross section of parotid gland )A)Five year age showed low intensity of VEGF(brow color) (B)One year age showed high intensity of VEGF(brown color).Immuohistochemistry(X40).

### Discussion

The present study showed that parotid salivary gland consist of spherica serous acini connected with branched ducts. These result coincide with <sup>8</sup> in buffalo; <sup>9</sup> in goat and sheep ; <sup>10</sup> in rhesus monkeys; <sup>11</sup> in barking

deer; <sup>12</sup> in human and rodent; <sup>13</sup> in local rabbit; <sup>14</sup> in rat; <sup>15</sup> in mice and <sup>16</sup> in rabbits. These result disagreed with <sup>17</sup> in castrated donkey; <sup>18</sup> in dog; <sup>19</sup> in mammals; <sup>20</sup> in dog, cat and ferret, who observed that parotid gland was a mixed gland serous and mucous.

There were intercalated ducts which attached with striated ducts. These results agree with study of <sup>21</sup> in the African palm squirrel, who reported the presence of a large number of intercalated duct in the parotid gland with only serous cells. These ducts involved the intra lobular ducts. There were larger ducts named interlobular ducts reported by <sup>22</sup> in camel; <sup>17</sup> in castrated donkey and <sup>8</sup> in buffalo, who reported the intercalated duct and striated duct were referred to intralobular duct, disagree with <sup>23</sup> in buffalo who reported that the intralobular ducts in buffaloes without striated duct.

There were myo epithelial cells located between the basal membrane and secretory acini and ducts agree with study of <sup>24</sup> in Hungarian hamster, disagree with <sup>25</sup> in rat who reported that, the serous acini lack myoepithelial cells.

The intensity of connective tissues were increased in the parotid salivary glands with age of five years as compared to the parotid salivary gland with age of one year agree with <sup>26</sup> and Scott and co-workers e.g., <sup>(27,28)</sup> in human; <sup>29</sup> in rats; <sup>30</sup> in human; <sup>31</sup> 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 <sup>32</sup> in human; <sup>33</sup> in rats.

There were a few fibrosis in the parotid salivary gland of five year age as compared with parotid salivary gland of one year which showed no any fibrosis, the result agree with <sup>1</sup> in human; <sup>34</sup> in human; <sup>35</sup> in rat.

The mean diameter of acini for parotid gland of five years age observed increased significantly  $p < 0.05$  ( $13 \pm 0.33$  micron) as compared to the parotid of one year age ( $10.4 \pm 0.02$  micron) increase in the diameter of both the serous and mucous acini has been shown in this study agree with <sup>36</sup> in human; <sup>31</sup> in rats; <sup>37</sup> in rats.

The mean diameter of intercalated duct for parotid gland of five years age observed increased significantly  $p < 0.05$  ( $40.13 \pm 2.61$  micron) as compared to the parotid of one year age ( $26.56 \pm 1.22$  micron) This observation agrees with <sup>38</sup> in rats; <sup>39</sup> in human, who stated that there is an increase in the ductal diameter with aging.

The intensity of PAS reaction (glycogen content) were decreased in the parotid salivary glands of five years

age as compared to the group of one year age agrees with <sup>40</sup> 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 results showed that the intensity of VEGF which showed as brown color were decreased in the parotid salivary gland of five year age as compared to the group of one year age agrees with <sup>(5,6)</sup> in rat, they observed that there were age-related decreases in the number of acinar cells, increase in eosinophilic zymogen granules in cells, collagen accumulation in fibrotic areas and dilatation in interlobular ducts. Also, while type I collagen and MMP-2 immunoreactivity were moderate in the salivary glands of the young mice, they were high in the salivary glands of the old mice.

**Conflict of Interest:** The author has no disclosures to report.

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**Ethical Clearance:** Not required.

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