Importance of calcium on the parotid salivary gland structure (experimental study on rats)

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Research Article

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Abstract

**Background** Calcium makes up 1-2 percent of a person's body weight, and 99% of it can be found in bones and teeth. The remaining circulates in the blood, muscles, and other tissues. Calcium deficiency diet affects general health by causing obesity, fat accumulation, diabetes (insulin resistance), osteoporosis and finally bad oral health due to the non-functioning salivary gland caused by hypocalcemia. If calcium deficiency is not treated, it is considered a fatal condition.

Calcium is used entirely by the salivary gland to induce its secretory function also it acts as a common modulator mediator for all receptors. Calcium deficiency increases fat cell formation and decreases exocytosis.

Aim of this study is to investigate the effect of calcium on general health and on the parotid salivary gland structure in rats.

**Methods:** twenty-four adult male albino rats weighting from 150-200 grams were divided into two groups 12 rat each, group I: control, group II: calcium deficient diet.

Body weight, general health observations were measured weekly throughout the experimental period while blood serum level of cholesterol, calcium, insulin, and glucose were all measured at the end of the experiment which lasted for 8 weeks.

At the end of the experiment, the rats were euthanized, and the parotid glands were dissected, prepared for light and electron microscopic examination.

**Results:** Group II showed obesity, increase in blood cholesterol, insulin, and glucose level. The ultrastructural and histological examinations showed loss of the normal architecture of acini and duct system as well as the capsule.

**Conclusion:** calcium deficient has a toxic effect on general health and on the parotid salivary gland structure.

1. **Introduction**

Calcium is the most abundant mineral in the human body, it is best known for its important role in bone health and protection from osteoporosis. However, in addition to its key role in imparting strength to bones and teeth, calcium plays a critical role as a messenger in cell-signaling pathways throughout the body and is necessary for normal cell function, transmission of nerve signals, secretion of hormones, blood coagulation, muscle contraction, and muscle relaxation[1, 2]

Calcium is stored in the smooth endoplasmic reticulum to be released when needed, calcium is also used by the nucleus and in the citric acid cycle of the mitochondria, it may be responsible for the cell attachment as it is needed in the structure and function of the adheren and tight junctions[3]
Calcium plays a critical role during salivary gland secretion, it helps the rough endoplasmic reticulum in protein folding, it is also utilized by the mitochondria for energy production which is essential for cell activity. Moreover, calcium is stored in the smooth endoplasmic reticulum to be released during salivary gland secretion. Calcium may be a common intracellular mediator for all salivary gland receptors, (β-adrenergic and acetyl choline receptor) as it modulates the activity of protein kinase A as well as the activity of protein kinase C. Low Ca diet affects the mechanism of protein kinase activity by lowering the PKA, PKC and CAMP thus lead to salivary gland dysfunction. [3, 4]

It also stimulates exocytosis but to a lesser degree than cyclic adenosine monophosphates (CAMP). Moreover, calcium stimulates the opening of the potassium channel, chloride channel and aquaporin channel. [5]

The Neurotransmitter-stimulation increases the cytosolic [Ca2+] in acinar cells which is the primary trigger for salivary fluid secretion from salivary glands. The loss of which is a critical factor underlying dry mouth conditions in patients. The increase in [Ca2+] regulates multiple ion channel and transport activities that together generate the osmotic gradient which drives fluid secretion across the apical membrane.[6, 7]

Moreover, calcium play an important role in the maturation of the secretory granules by concentrating secretory proteins through the addition of glycosaminoglycans and the formation of calcium bridges. In addition, the large and highly charged, mucin polymers also must undergo condensation and stabilization by interaction with calcium ions and positively charged organic molecules. Calcium also motivates the exocytosis of the secretory granules. As the elevated intracellular Ca++ levels promote fusion of the secretory granule leading to discharge.[3]

Calcium deficient diet decreases general health as it led to obesity by increasing lipogenesis and decreasing lipolysis as calcium in diet decreases adipocyte fatty acid synthase and inhibits the differentiation of preadipocyte [5]. Moreover, it can cause diabetes, hair loss and irritability as it disturbs the function of the nervous system causing nervousness. [8-11]

Hypocalcemia can destroy the oral health by alternating the salivary gland structure and function since calcium stimulates exocytosis by increasing the maturation, docking and secretion of the salivary gland granules thus hypocalcemia can therefore increase the number of immature secretory granules as well as decreasing exocytosis. Furthermore, calcium induces the opening of the potassium, chloride, and aquaporin channels so hypocalcemia can decrease the fluid secretion as well. [3]

Hypocalcemia accelerates the risk of insulin resistance (IR), as calcium can enhance pancreatic B cell function and increase insulin sensitivity, so any deficiency in calcium level may lead to decrease in insulin sensitivity which leads to hyperinsulinemia.[12]

Since studies performed on the effect of calcium deficient diet on the histological and ultrastructure of the parotid salivary gland and on the general body health are limited. That’s why the aim of the present
study was to evaluate the effect of calcium deficient diet on general health as well as on the structure of the parotid salivary glands.

2. Materials And Methods

Study design

Twenty-four male rats weighing 150-200 grams were used in this study. These animals were obtained from and caged in the animal house in Faculty of medicine, Alexandria University, after gaining the approval of the Research Ethics committee of the faculty of Dentistry, Alexandria university (IORG0008839), they were caged in specially designed wire mesh cages.

Group I: (Control group n=12) the animals were maintained on normal rat chow, normal calcium content (1.1-1.3%) [13, 14]

Group II: (calcium deficient diet group n=12) They were fed calcium deficient diet containing 0.1-0.3% calcium.[14]

Percentage of calcium in food: normal calcium composition is 1.1-1.3% and was decreased to 0.1-0.3% in calcium deficient diet group. [14, 15]

Outcome measures:

2.1-General health: [16]

Any abnormal behavior such as changes during handling, changes in activity, any decrease in food intake or water intake or hair loss was evaluated.

2.2-Body weight: [16]

The weight of each rat in both groups was measured every week till the end of the experiment which lasted for 8 weeks.

2.3-Biochemical analysis: [15]

Blood was obtained from the rats tail vein under anesthesia, serum was isolated by centrifugation (3000 rpm, 15 min) and frozen at -80 °C. Serum cholesterol was measured using routine laboratory methods, fasting blood insulin level was qualified using rat specific insulin, ELISA kits (enzyme linked immunoassay). Serum glucose was measured using an auto analyzer, calcium concentration was measured using an auto analyzer all of them were obtained after scarification.

2.4-Histological procedure:[15]
Parotid salivary glands were fixed in 10% neutral-buffered formalin, washed, dehydrated with ascending concentrations of ethanol, cleared with xylene, and embedded in paraffin wax blocks. Sections were cut at a thickness of 4 μm and stained with Hematoxylin and Eosin then examined by light microscope.

2.5- Ultra structural specimen preparations: [17]

Specimens were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 mol/l PBS (phosphate buffer solution) at pH 7.4 for 2 h and then postfixed in 1% osmium tetroxide in the same buffer for 1-4 h at 4°C. The specimens were processed and embedded in Epoxy resin in BEEM capsules at 60°C for 24 h. Ultrathin sections were obtained using a glass knife in an ultramicrotome. Each section is only 50-100 nm thick stained with uranyl acetate and lead citrate, and examined by Transmission electron microscope (Tokyo, Japan) in the Electron Microscope Research unit at faculty of science Alexandria university (Alexandria, Egypt).

2.6-Data management and statistical analysis: [18]

Results obtained from blood analysis and body weight were tabulated and analyzed using ANOVA test to compare between the two groups.

3. Results

3.1 Clinical observations

Changes in body weight

Fig 1a showed that there was statistically significant increase in the mean weight gain throughout the experiment between calcium deficient diet group and control (p1=0.00759)

Hair loss

The amount of hair covering the rat's skin was clinically observed daily during the whole eight weeks, we found that there were patches of uncovered skin in rats of group II during the last two weeks in comparison with the control.

Behavioral changes

There were daily observations for any abnormal behavior during the eight weeks, it was noted that in the last three weeks the rats in the calcium deficient diet group appeared nervous and irritable, hitting their heads in front of the cage's walls.

Changes in the activity

Changes in the activity were observed three times daily- morning, afternoon, and night for an hour calculating the number of movements they did, it was found that there was a decrease in the activity of
calcium deficient diet group in comparison with the control group during the last three weeks.

**Amount of food intake**

*Fig1b* showed that there was a statistically significant increase in the mean amount of food intake between calcium deficient diet group and control (p1 = 6.90174E-05).

**Amount of water intake**

*Fig 1c* showed that there was a statistically significant increase in the mean of amount of water intake between calcium deficient diet group and control (p1 = 4.66086E-05).

3.2 serological analysis

**a) Cholesterol level**

*Fig 2a* showed that the Calcium deficient diet group had a significant increase in cholesterol serum level than the control group (P1 = 0.001829599).

**b) Calcium serum level**

*Fig2b* revealed that the calcium deficient diet group had a statistically significant decrease in the serum calcium level in comparison with the control group (p1 = 0.000845807).

**c) Insulin in blood level**

*Fig 2c* showed that there was a significant increase in the insulin blood level in calcium deficient diet group (p1 = 2.47567E-06).

**d) Blood glucose level**

*Fig 2d* revealed that there was no significant difference in the blood glucose level in calcium deficient diet group (p1 = 0.338848214).

3.3 light microscopic results

**Control group**

Histological analysis was performed from the histological images of the control group (Fig 3). The evaluation of the different structure in the parotid salivary gland showed the Well-developed connective tissue capsule that contains fat cells, blood vessels and cells (Fig 3a). Normal architecture of the serous acini with spherical nuclei and normal cytoplasm with typical degree of stainability. Normal intercalated duct lined by cuboidal cells with rounded centralized nucleus (Fig 3b). The secretory straited ducts lined by tall columnar cells with well-defined basal striations (Fig 3c). Normal excretory duct located in the
connective tissue septa lined by pseudostratified columnar epithelium surrounding a wide lumen with some stagnated saliva and surrounded by connective tissue fibers and cells (Fig3d)

**Calcium deficient diet group**

This group showed abnormal accumulation of fat cells, inflammatory cells and thickening in connective tissue fibers (Fig 4a). Atrophy in the acini with numerous vacuolation of the cytoplasm and pyknotic nuclei. Also, the intercalated duct showed degeneration in the epithelial lining (Fig 4b). The secretory striated duct revealed widening in the lumen with loss of their striations and associated with dilated blood capillary engorged with RBCs, moreover degeneration of the epithelial lining of the duct (Fig 4c). The excretory duct exhibited an abnormal widening of the lumen associated with stagnated salivary secretion. Degeneration of the epithelial lining and connective tissue fibrosis surrounding the duct (Fig 4d).

**3.4 Ultrastructural results**

**Control group**

The ultrastructural results confirmed the Light microscopic findings. The control group exhibited part of a normal euchromatic nucleus with regular nuclear membrane, striking amount of well-organized rough endoplasmic reticulum in the cytoplasm of the serous cell and normal mitochondria (Fig 5a-b) Also mature electron dense secretory granules were seen (Fig 5b). Normal intercalated duct formed of cuboidal cells with rounded centrally located euchromatic nuclei and little electron dense secretory granules apically surrounding a narrow lumen (Fig 5c). The intercalated duct also showed well defined junctional complex (Fig 5d). Normal secretory striated duct lined by tall columnar cells with normal euchromatic nuclei, well organized basal infoldings, with normal lumen. They were associated with a nearby blood capillary containing RBCs (Fig 5 e,f). accommodating large number of longitudinal oriented mitochondria with the nucleus containing well developed nucleolus. (Fig 5f)

**Calcium deficient diet group**

The ultra-histological analyzes were performed from the results obtained by the electron microscopic examination (Fig 6). The evaluation of the different structures and organelles in the parotid salivary gland showed a part of a serous cell having heterochromatic degenerated nucleus with irregular nuclear membrane, numerous dilated rough endoplasmic reticulum, and swollen Golgi complex, associated with degenerated mitochondria (Fig 6a, b). Separation between the adjacent cells were also seen (Fig 6b). Moreover, an abnormal intercalated duct with partially degenerated epithelial lining and slight widening of the lumen. The electron dense heterochromatic nuclei and vacuolation in the cytoplasm of the duct were also observed (Fig 6c) In addition, there was dilatation in the rough endoplasmic reticulum RER and degeneration in the mitochondria (Fig 6d). The secretory striated duct had a huge wide lumen with loss of its basal infoldings along with scattered degenerated mitochondria. (Fig 6e, f)
4. Discussion

Calcium is a mineral most widely associated with healthy bones and teeth, although it usually plays an important role in blood clotting, helping muscles to contract, and regulating normal heart rhythms and nerve functions. About 99% of the body's calcium is found in bones, and the remaining 1% is found in blood, muscle, and other tissues.[19, 20]

Hypocalcemia is caused due to insufficient amount of calcium in diet. In this case of such deficiency, the body will pull calcium from the bones to maintain normal blood calcium levels leading to osteoporosis. [21] Moreover, hypocalcemia accelerates the risk of insulin resistance (IR) due to its inability to improve insulin sensitivity, so calcium decreases the risk of diabetes type 2 by increasing insulin sensitivity.[22, 23] In addition, hypocalcemia can increase lipogenesis and decrease lipolysis causing obesity.[8]

On the other side hypocalcemia can disturb the general health by causing physical inactivity, irritability and weakness in nails and hair.[24] Also, hypocalcemia can lead to bad oral health due to decrease in the salivary glands secretions and function.[6]

Saliva serves some protective functions in respect of the oral mucosa and gingiva, the salivary glands have an important function related to oral health maintenance but also, they are associated with other metabolic disturbances such as the occurrence of diabetes type 2 caused by insulin resistance from low calcium diet.[25]

The food and drug administration (FDA) guidelines have appropriately designed the need for rat experimentation in the preclinical evaluation of agents used in the prevention or treatment of obesity. Male rats were used to exclude any hormonal changes that may disturb the results of calcium deficient diet. [26] Eight weeks was enough to induce obesity in rats fed with calcium deficient diet and to increase the oxidative stresses that leads to insulin resistance which is known as prediabetic stage.[22]

We have chosen the parotid glands as it seems to be more affected to the destruction caused by calcium deficient diet, the oxidative damage to the parotid glands induces insulin resistance, this is related to their physiological morphology, namely the presence of many adipocyte cells in the parenchyma of the parotid glands and the adipocyte tissue are the main source of ROS that leads to IR(insulin resistance). [27]

Furthermore our study concluded that low calcium diet leads a significant increase in body weight in comparison to the control group this was in agreement with other study which found that Low dietary calcium intake stimulates high levels of PTH and 1,25-hydroxy vitamin D, which stimulates high levels of intracellular calcium in adipocytes leading to lipogenesis and inhibiting lipolysis. [28]

The current study illustrated that the Calcium deficient diet causes physical inactivity in the last three weeks this is because Calcium helps muscles contract and relax. Muscles lacking calcium can no longer maintain their normal tone. This can lead to aches, cramps, spasms, and muscle weakness[29]
The present study showed that Calcium deficient diet causes hair loss in the last two weeks, this was in coincidence with other study that explained the importance of calcium for strong, healthy hair as it aids hormone and enzyme secretion, including the androgen hormones and biotin enzymes involved in healthy hair growth. It can also help to absorb iron, which is vital for strong, healthy hair [9]

In the calcium deficient diet group the rats were nervous and irritable in the last three weeks as Calcium ions are critically important in many functions of the nervous system from neurotransmitter release to intracellular signal transduction.[30]

The present study revealed that the Calcium deficient diet group causes increase water intake. In the prediabetic stage water consumption increases as kidneys is forced to work overtime to filter and absorb the excess glucose. When the kidneys can't keep up, the excess glucose is excreted into urine, dragging along fluids from the tissues causing dehydration. [31]

Moreover, the current study demonstrated that Calcium deficient diet causes increase food consumption, This is because when the body doesn't absorb enough blood sugar as in the prediabetic stage due to insulin resistance, the body doesn't get as much energy from each snack or meal, this causes an increase in the feel of hunger [31]

The serological results obtained in this study revealed that the serum calcium level was significantly decreased in the calcium deficient diet group compared to the control group. Generally sufficient Ca gets ingested through the normal diet. It is absorbed from the upper intestinal tract to the circulatory system that's why any changes in the amount of calcium in diet affects its amount in blood.[32]

Calcium deficient diet group showed a significant increase in serum cholesterol level which is in accordance with other study that explains that the calcium is known to bind with bile acids to form insoluble soaps and thus presumably can remove cholesterol entering the gut via the enterohepatic circulation. [33]

The results of the current study revealed that animal groups fed with calcium deficient diet showed a significant increase in blood insulin level as calcium can enhance pancreatic B cell function and increase insulin sensitivity. [12]

The results of the study revealed that there was a non-significant increase in the blood glucose level in calcium deficient diet. Oral Calcium Supplementation Reduces Insulin Resistance therefore any deficient in calcium may lead to insulin resistance and therefore elevated blood glucose level. [34]

The current histological results of the calcium deficient diet group revealed a significant increase in the amount of adipocyte cells in the capsule and in the interlobular Septa. These results support that hypocalcemia can increase lipogenesis and decrease lipolysis as calcium in diet decreases adipocyte fatty acid synthase.[8]
Additionally the histological observations seen in the calcium deficient diet group showed some degeneration in the serous acini cells along with the lining of the excretory duct characterized by the present of vacuoles in the acini and thinning in the lining of the excretory ducts, the chronic inflammation caused by adipocyte state leads to generation of oxygen free radical by the monocyte and neutrophils from their NAPDH oxidase enzyme activity that is activated during phagocytosis those free radicals were implicated in the degenerations occurred inside the salivary gland [35]

The current results revealed a significant increase of fibers in the connective tissue septa and around the interlobular ducts and the intralobular ducts, this is because chronic inflammations cause tissue destruction and leads to progressive fibrosis. Inflammation and fibrosis can thus be viewed as a continuum of events within the framework of tissue defense, repair and regeneration. [36]

The existing histological results showed an extensive dilatation of the ductal system, stagnation of saliva and congestion of blood vessels all these histological results are consequences of salivary gland dysfunction, Garant PR discussed in his book that dilatation of the ducts could be attributed to accumulation of the salivary secretion and failure of exocytosis, congestion of blood vessels could be attributed to Passive hyperemia in which blood can't properly exit an organ, so it builds up in the blood vessels.[3]

The Ultrastructural results showed dilatation and degeneration in the membrane of the rough endoplasmic and Golgi apparatus. this is because any Loss of luminal Ca2+ in the rER causes rER stress which activates an unfolded protein response, which, depending on the duration and severity of the stress, can reestablish normal rER function or lead to cell death. As a consequences during rER stresses there is an increase of calcium release from ER which enter the mitochondria causing calcium accumulation that lead to rupture of the mitochondrial membrane and release of pro-apoptotic enzymes. [37]

Moreover, the current study found that inflammatory process induces oxidative stress and reduces cellular antioxidant capacity this leads to Overproduction of free radicals which react with the lipids and proteins that form the membranes surrounding these organelles causing its destruction. In addition to this, these free radicals can lead to mutation and damage to the DNA which causes degeneration in the nucleus as shown in our results. [35]

In addition, calcium is important for the nucleus. As Nuclear Ca2+ plays a significant role in regulating the transcription factor CRE-binding protein and its coactivator CREB-binding protein (CBP) moreover, nucleus Ca2+ was shown to bind to and directly regulate DNA structure. [38]

Furthermore, we found a significant separation between the acinar cells, this explains the importance of calcium in the adherens and tight junctions, as in tight junction increasing the intracellular calcium by disrupting the intracellular stores due to decrease of calcium in the cytoplasm, may interferes with tight junction formation. This indicates that regulation of intracellular calcium is critical for normal function of
tight junction. Moreover, Binding of Ca2+ to each Extracellular domain of the adherens junction is required for the correct conformational organization of the cadherin extracellular domain [39]

On top of that, there was an increase in the immature secretory granules, this was agreed with Garant PR who discussed that Calcium may be a common intracellular mediator for all salivary gland receptors. (β-adrenergic and acetyl choline receptor) as it modulates the activity of protein kinase A as well as the activity of protein kinase C. It also stimulates exocytosis, docking and maturation of the secretory granules. The formation of secretory granules involves a maturation process requiring the condensation of secretory proteins, the mechanisms for concentrating secretory proteins include addition of glycosaminoglycans and the formation of calcium bridges.[3]

5. Conclusion

Calcium deficient diet can affect the general health by causing obesity, diabetes type 2, hair loss, laziness and irritability, it can also decrease the oral health by decreasing the salivary secretions and alternating the parotid salivary gland structure.

6. Recommendations

Further studies should be done to see the effect of calcium deficient on the other salivary glands and its effect on dental (pulp, dentin, cementum) and paradental tissue (PDL, bone, gingiva, oral mucosa)

Abbreviations

PBS (phosphate buffer solution), CAMP (cyclic adenosine monophosphates), ELISA kits (enzyme linked immunoassay). insulin resistance (IR).

Declarations

Authors contribution

All authors contributed to the conception and the design of the study, Maha Montaser and Sara Ashraf acquired the data, Amel El Hak and Gehan Alba analyzed and interpreted the data. Mounir Eladawy prepared the diet. all authors revised the article and read and approved the final article.

Funding information: the research was developed without funding.

Compliance with ethical standards

Conflict of interest: all authors declares that they have no conflict of interest.

Ethical approval: the research protocol was approved by the ethics committee of the Research Ethics Committee, Faculty of Dentistry, Alexandria University. And all the applicable international, national,
and/or institutional guidelines for the care and use of animals were followed.

**Informed consent:** for this type of study, formal consent is not required

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Figures
Figure 1

Graphical representation between the two relative groups control and low calcium diet showing:

(a) Bar chart regarding the mean of body weight in grams.

(b) Bar chart regarding the mean of amount of food intake in grams.
(c) Bar chart regarding the mean of water intake in ml.

Figure 2

Graphical representation between the two relative groups control and low calcium diet group showing:

(a) : Bar chart regarding the mean of cholesterol blood level in mg/dl.

(b) : Bar chart regarding the mean of calcium in blood in mg/dl.

(c) Bar regarding the mean of insulin in blood Ng/ml.

(d) Bar chart regarding the mean of glucose in blood mg/dl.
Figure 3

Light micrograph (L.M) of control group showing:

a) Normal histological architecture of the parotid salivary gland consisting of serous acini (AC) surrounded with a well-developed connective tissue capsule (CT capsule) that contain fat cells, blood vessels and connective tissue cells [H&E Stain, original magnification × 100] b) Normal histological
architecture of the parotid salivary gland consisting of serous acini, the acinar cells are pyramidal in shape surrounding a narrow lumen. Normal secretory straited duct lined by columnar epithelium, and basal striations (arrow) c) Normal serous acini and intercalated duct with narrow lumen lined by cuboidal cells (arrow) d) Normal excretory duct (arrow) located in the connective tissue septa and lined by pseudostratified columnar epithelium with some stagnated saliva and surrounded by connective tissue fibers and cells [H&E: original magnification ×400]
Figure 4

Light micrograph (LM) of calcium deficient diet group showing:

(a) Abnormal accumulation of fat cells (FC), inflammatory cells and thickening in connective tissue fibers (CT fibers) in the connective tissue capsule [H&E Stain, original magnification × 100]

b) Atrophy in the acini with numerous vacuolation of the cytoplasm and widening of the lumen of the striated duct and loss of its striation (arrowhead). Note the degeneration of the intercalated duct (arrow)

c) Loss of the normal structural integrity of the acini with numerous vacuolization of their cytoplasm. Widening in the lumen of the secretory striated duct with loss of their striations (arrowhead) and associated with dilated blood capillary with engorge RBCs (BC),

d) Excretory duct (ED) with abnormal widening of the lumen associated with stagnated salivary secretion. Note degeneration of the epithelial lining and connective tissue fibrosis surrounding the duct [H&E Stain, original magnification × 400]
Figure 5

Transmission electron micrograph showing the parotid gland of the control group.

(a) A part of a normal euchromatic nucleus(n) with regular nuclear membrane, striking amount of well-organized rough endoplasmic reticulum (rER) in the cytoplasm of the serous cell and normal mitochondria(m) (original magnification× 6000) b) A part of a serous acinus with well-formed normal
euchromatic nucleus (n) containing a well-defined nucleolus (nu), few mature electro dense secretory granules (SG) and well developed rough endoplasmic reticulum (rER). Note: normal mitochondria (m) (Original magnification × 5000)

c) Normal intercalated duct formed of cuboidal cells with rounded centrally located euchromatic nuclei (n) surrounding a narrow lumen (Lu) (Original magnification × 1500)

d) Higher magnification of the previous inset (Fig5c) showing euchromatic nucleus (n) with regular nuclear membrane, well defined junctional complex (JC) and secretory granules (SG) (original magnification × 2500)

e) A normal secretory striated duct lined by tall columnar cells with normal euchromatic nuclei (n), normal basal folding (black arrow), with normal lumen (Lu) Note the nearby blood capillary containing RBCs (BC) (original magnification × 800)

f) High power view of the previous micrograph inset (Fig5e) revealing: Normal basal in folding (white arrow) accommodating large number of longitudinal oriented mitochondria (m) Note, the nucleus contains well developed nucleolus (nu) (original magnification × 2500)
Figure 6

Transmission electron micrograph of the parotid gland of the control group:

(a) A part of a serous cell having heterochromatic degenerated nucleus (n) with irregular nuclear membrane, numerous dilated swollen rough endoplasmic reticulum (rER) and swollen Golgi complex (GC) associated with degenerated mitochondria (m). Note, dramatic dissolution in the cytoplasm (original
magnification×5000) b) A part of a serous secretory cell with degenerated apoptotic nucleus and heterogenic chromatin(n), dilated Golgi complex (GC) and rough endoplasmic reticulum(rER), degenerated mitochondria(m) combined with multiple immature secretory granules(SG) Note: separation between the adjacent cells(original magnification×8000)c) An abnormal intercalated duct with partially degenerated epithelial lining and slight wide lumen (Lu). The epithelial lining disorganized and degenerated organelles, electron dense heterochromatic nuclei (n) and a vacuole in the cytoplasm (V)(original magnification × 4000) d) High power view of the previous micrograph inset(Fig6c) showing: pyknotic heterochromatic nucleus(n) and few mitochondria(m) with loss of their structural details. Note, dilated RER (Original magnification× 5000) e) A secretory striated duct with a huge widening in its lumen (Lu), loss of the the basal folding(arrow) associated with scattered degenerated mitochondria(m) which loses its crista (original magnification×1200) f) High power view of the previous micrograph inset (Fig6e) showing loss of the basal folding (arrow) and massive number of scattered degenerated mitochondria(m) with dissolution of the (DC) cytoplasm with few secretory granules (original magnification×2500)