Saliva and Oral Health

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Introduction

Saliva is an important fluid that play an essential role in maintaining the integrity of the oral structures and the human body as whole (Puy, 2006; Mese and Matsuo, 2007; DeAlmeida et al, 2008). It is considered a unique complex and important body fluid that contains a number of systems which serves a wide spectrum of physiological needs to protect the oral mucosa and the whole body from infection (Ten Cate, 2003; Harris and Godoy, 2004).

Saliva through its flow rate play an important role in maintaining the integrity of the soft and hard tissues in the oral cavity (Stooky, 2008). It has a complex physical and chemical composition through which it can perform a considerable number of protective functions to the oral cavity as well as to the body (Rehak et al, 2000; Ten Cate, 2003; Harris and Godoy, 2004; Amerongen et al, 2007). Several factors influencing the salivary flow and its composition, these factors vary greatly among individuals and in the same individual under different circumstances (Edgar and Dawes, 2004).

In this review of literature details of saliva concerning physical and chemical properties of saliva are going to be discussed in relation to oral health disease.

1.1 Definition of saliva

Saliva is a watery substance that is produced in the oral cavity of human and constitutes about 98 % of water, while the remaining 2 % are electrolytes, glycoprotein and antibacterial component such as certain types of immunoglobulins and lysozyme enzyme (Fejerskov, 2008).The oral fluid is an aqueous fluid composed of a complex mixture of secretory products (that are organic and inorganic products) from salivary glands and other substances that are coming from the oropharynx, upper airway, gastrointestinal reflux, gingival sulcus fluid, food deposits and blood derived compounds (Dodds et al, 2005). The salivary fluid is considered to be an exocrine secretion (Ferraris et al, 2006).

A total volume of about 1-1.5 litters of saliva is produced daily in the oral cavity of healthy adult and this amount of secreted saliva spread to an area of about 200 cm² in the oral cavity resulting in a thin film of about 10-100 mm in thickness (Humphery and Williamson, 2001).
1.2 Salivary glands

Salivary glands in mammals are exocrine glands, with ducts, that produce saliva into the oral cavity and they are divided into major salivary glands (parotid, submandibular and sublingual gland) as shown in Figure (1-1), and hundreds of minor salivary glands distributed in different sites of the oral cavity (Ten Cate, 2008).

1.2.1 Major salivary glands

1.2.1.1 Parotid gland

The paired parotid glands are the largest of the salivary glands, they are found overlying the mandibular ramus and anterior and inferior to the external ear and occupy the parotid facial spaces (an area posterior to the mandibular ramus, anterior and inferior to the ear on each side of the head), it extends the head irregularly from the zygomatic arch to the angle of the mandible and can be effectively palpated bilaterally by starting anterior to each ear, moving to the cheek area and then inferiorly to the angle of the mandible and open out through Stensen’s duct adjacent to maxillary second molar (Steven, 2009).

Above the parotid gland lies the external auditory meatus and tempromandibular joint. Below the parotid overlaps the posterior belly of the digastric muscle medially lies the styloid process posteriorly, the parotid over flows the sternocleidomastoid and anteriorly it overlies the mandible with the overlying masseter muscle (Fehrenbach, 2007).

Branches of the external carotid artery traverse the glandular tissue and supply the parotid gland with oxygenated blood, the main branch to supply the gland is the transverse facial artery, whereas numerous local veins drain the organ these veins drain into tributaries of the external and the internal jugular vein (Bucur, 2011). The maxillary and superficial temporal veins meet to form the retro mandibular vein within the parotid gland, but are not responsible for draining it (Costanzo, 2006) as shown in Figure (1-2).

Although the facial nerve passes through the parotid gland, it does not receive innervation from it. Instead, the parotid gland is innervated by parasympathetic fibers that arise in the inferior salivatory nucleus, travel with the glossopharyngeal nerve and lesser petrosal nerve to the otic ganglion, where they synapse and then continue with the auriculotemporal nerve to the parotid gland (Totan, 2011).

1.2.1.2 Submandibular gland

This second major salivary gland located beneath the floor of the mouth lying superior to the digastric muscle divided into the superficial and deep lobes which separated by mylohyoid muscle (Moore et al, 2010) as shown in Figure (1-3).

Secretions are delivered into the Wharton’s ducts on the deep portion after which they hook around the posterior edge of the mylohyoid muscle and proceed on the superior surface laterally. Ducts are then crossed by the lingual nerve lingual nerve, and ultimately drain into the sublingual caruncles (carunculasublingualis) on either side of the lingual frenulum along with the major sublingual duct (Bartholin duct) (Bruce, 2010).

Secretions of submandibular salivary gland, like the secretions of the other salivary glands, are regulated directly by the parasympathetic nervous system and indirectly by the sympathetic nervous system, where the parasympathetic innervation to the submandibular glands is provided by the superior salivatory via the chorda tympani which is a branch of the facial nerve, that becomes part of the trigeminal nerve and lingual nerve prior to synapsing on the submandibular ganglion thus increase parasympathetic activity promotes the secretion of saliva (Keith, 2010). The sympathetic nervous system regulates submandibular secretions through the arteries that supply it thus increased the sympathetic vasoconstriction activity reduces glandular blood flow, thereby decreasing the volume of the salivary secretions, producing an enzyme rich mucous saliva. Nevertheless, direct stimulation of sympathetic nerves will cause an increase in salivary enzymatic secretions (Koeppen, 2010).

1.2.1.3 Sublingual gland

They are the smallest major salivary glands in the oral cavity lies anterior to the submandibular gland under the tongue, beneath the mucous membrane. They are drained by 8-20 excretory ducts called ducts of Rivinus. The glands open through a large ducts called Bartholin’s ducts that open with Wharton's Ducts at the sublingual caruncle. Also they have small ducts open separately in to the mouth on an elevated crest of mucous membrane (Blanco, 2005). Chorda tympani
which is branch of facial nerve is the secretomotor to the sublingual gland (Shivnani, 2005).

Figure (1-1): Major salivary glands. (Gray's Anatomy, 2008).
Figure (1-2): Drawing shows the major blood vessels in the area of salivary glands. (Gray's Anatomy, 2008).

1 - Retro mandibular vein.  2 - External carotid artery.
3 - Facial artery and vein  4 - Lingual artery and vein.
5 - External carotid artery  6 - Internal jugular vein.
7 - External jugular vein.

Figure (1-3): Parotid, submandibular and sublingual salivary glands. (Gray's Anatomy, 2008).
1.2.2 Minor salivary glands

As mentioned before there are a hundreds of minor salivary glands scattered throughout the oral cavity, the most important of these glands are labial and buccal glands, glossopalatine glands, palatine glands, and lingual glands (Ten Cate, 2008).

Labial and buccal glands are located in lips and cheeks and are of seromucous type, glossopalatine glands are purely mucous glands and they are located in the isthmus of the glossopalatine fold, palatine glands are also purely mucous glands, they consist of hundreds of glandular aggregations in the lamina propria of the postero lateral region of the hard palate and in the submucosa of the soft palate and the uvula, where as lingual glands can be divided into two groups, anterior group near the apex of the tongue and are of the mucous character, and posterior group that are of seromucous type, their ducts open on the ventral surface of the tongue near the lingual frenum (Baurmash, 2003).

Most of the minor salivary glands receive parasympathetic innervation from the lingual nerve, except for the minor glands of the palate, which receive their parasympathetic fibers from the palatine nerve, fed by the Sphenopalatine ganglion (Carlson, 2004).

1.3 Histology of salivary glands

In general salivary glands can be classified according to prevailing type of secretory cells into serous (protein – secreting) such as parotid gland, mucous (mucin – secreting) and mixed (seromucous) glands such as submandibular gland that produce serous and mucous secretions but predominantly serous secretions, and sublingual gland that also produce serous and mucous secretions but predominantly mucous secretions.(Pedersen and Bardow, 2002).

In salivary glandular tissues the main working parts consist of the branched ductal system and secretory end pieces called acini that is surrounded by myoepithelial cells as shown in Figure (1-4), these cells assist in propelling the secretion into the ductal system , the arrangement of cells in the end piece differ between serous and mucous glands, in that it tends to be arranged in a roughly spherical form in serous glands, while in mucous glands it tends to be arranged in a tubular configuration with a larger central lumen as shown in Figure (1-5) and (1-6), but in both types of glands the intercellular spaces between the cells in the end piece open into the lumen and this is the beginning of the ductal system (Ross and Romrell,1995). The salivary glands consist of three types of ducts, these are the intercalated ducts that have a low cuboidal epithelium and a narrow lumen, striated ducts that are lined by more columnar cells with many mitochondria , and excretory ducts that containing cuboidal cells and the terminal part is lined by stratified squamous epithelium, the fluid first passes through the intercalated duct, then it enters the striated duct and finally the saliva passes through the excretory duct (Kontis and Johns, 1998).

The end piece may contain serous cells, mucous cells, or a mixture of both, and the salivary glands may consist of a varied of these of these types of end pieces (Silvers and Som, 1998).

Salivary glands and their nerves and blood supply are supported by a connective tissue stroma (Sinha, 1999).
1.4 General functions of saliva

**Taste:** the salivary fluid initially formed inside the acini is isotonic with respect to plasma, however, as it runs through the network of ducts, it becomes hypotonic, the hypotonicity of saliva (low levels of glucose, sodium chloride, and urea) and its capacity to provide the dissolution of substances allows the gustatory buds to perceive different flavors (Berkovitz, 2002).

**Protection and lubrication:** a number of protective functions which are part of the total body's ability to maintain homeostasis can be performed via the complex physical and chemical composition of salivary secretion (Amerogen, et al, 2007). Saliva forms a seromucosal covering that lubricates and protects the oral tissues against irritating agents, this occurs due to mucins (proteins with high carbohydrate content) responsible for the lubrication, protection against dehydration, and maintenance of salivary viscoelasticity they also selectively modulate the adhesion of microorganisms to the oral tissue surface which contributes to the control of bacterial and fungal colonization in addition, they protect these tissues against proteolytic attacks by microorganisms (Nagler, 2004).
**Dilution and cleaning:** sugars in their free form are present in total stimulated and unstimulated saliva at a mean concentration of 0.5 to 1 mg/100mL (Lucasand Roberts, 2005). Salivary fluid tends to eliminate the excess of carbohydrates, thus, limiting the availability of sugars to the biofilm microorganisms. The greater the salivary fluid, the greater the cleaning and diluting capacity; therefore, if changes in health status cause a reduction in salivary fluid, there would be a drastic alteration in the level of oral cleaning (Nagler, 2004).

**Buffer capacity:** buffer is the solutions that tends to maintain a constant pH when acid or base is added, the buffer capacity is related to its concentration (Macdonald and Chaney, 2007). Hydrogen ions present in saliva via their secretion through salivary glands in form of inorganic and organic acids, produced by the oral microbiota and taken by food or acid drinks with an average pH of mixed saliva 6.7 and it is vary with salivary flow rate from 5.3 with low flow to 7.8 with peak flow (Tenovuo and Lagerlof, 1994; Bardow et al, 2000; Myers, 2010).

Regulation of oral pH is an important function of salivary buffering systems which are: bicarbonate, phosphate and proteins. The role of saliva includes not only supply calcium and phosphate ions to teeth but also buffering capacity to neutralize acids produced by the metabolism of hydrocarbons by bacteria in oral cavity to prevent demineralization of teeth and stop the formation and progression of dental caries (Takagi et al, 2004).

Bicarbonate is the most important buffering system but only at high flow rates (stimulated saliva) which is based on equilibrium relation between carbonic acid and bicarbonate. When an acid (H$^+$) is added, bicarbonate (HCO$_3^-$) release a weak carbonic acid (H$_2$CO$_3$), this rapidly decompose to water (H$_2$O) and carbon dioxide (CO$_2$). Since the mouth is an open system; carbon dioxide will be lost to the atmosphere, this will lead to more carbonic acid production and more carbon dioxide escape and increase in the binding of bicarbonate and hydrogen ions till a complete removal of the acid (Bradow et al, 2000; Takagi et al, 2004; Ericsson and Barthal, 2007).  

\[
\text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2
\]

Phosphate buffer system has an important role at low salivary flow rate (unstimulated saliva) and makes minor contribution to the total salivary buffer capacity and is mainly based on the ability of secondary phosphate ion (HPO$_4^{2-}$) to bind a hydrogen ion and form primary phosphate (H$_2$PO$_4^-$) ion. Protein buffer system has been regarded as insignificant or at least of minor importance and it based on containing H$^-$ binding sites (Tenovuo and Lagerlof, 1996).

Urea is considered as a fourth buffering system in saliva (Fakhoury and Peraldi, 1996) that many plaque bacteria possess urease enzyme which catalyses the conversion of urea to ammonia and carbon dioxide. Since ammonia is highly alkaline (contain hydrogen binding site), so it neutralizes acid and causes pH rise (Shu et al, 2007).

**Integrity of tooth enamel:** saliva plays a fundamental role in maintaining the physical-chemical integrity of tooth enamel by modulating remineralization and demineralization. The main factors controlling the stability of enamel hydroxyapatite are the active concentrations of calcium, phosphate, and fluoride in the salivary pH (Axelsson, 2000). The availability of calcium and phosphate ions in saliva can enhance remineralization of carious tooth before cavitation occurs (Stack, 2001). The presence of fluoride in saliva, even at physiologically low levels, is decisive for the stability of dental minerals (Humphrey and Williamson, 2002).

**Digestion:** saliva is responsible for the initial digestion of starch, favoring the formation of the food bolus (Costanzo, 2004). This action occurs mainly by the presence of the digestive enzyme $\alpha$-amylase (ptyalin) in the composition of the saliva this enzyme divide the starch into maltose, maltotriose, and dextrins, so this is considered to be a good indicator of properly functioning salivary glands (Vissink, 2008). This enzyme contributing 40% to 50% of the total protein produced by the glands, the greater part of this enzyme (80%) is synthesized in the parotids and the remainder in the submandibular glands, its action is limited to mouth because it is inactivated by the acid portion of gastrointestinal tract (Douglas, 2002).

**Tissue repair:** a tissue repair function is attributed to saliva since clinically the bleeding time of oral tissues appears to be shorter than other tissues, when saliva is experimentally mixed with blood, the coagulation time can be greatly accelerated (although the resulting clot is less solid than normal), as experimental studies in mice have shown wound contraction is significantly increased in the presence of saliva due to the epidermal growth factor it contains which is produced by the submandibular glands (Ten Cate, 2003).
Antimicrobial activity: saliva contains a spectrum of immunologic and non-immunologic proteins with antibacterial properties, some of these proteins are necessary for inhibiting the spontaneous precipitation of calcium and phosphate ions in the salivary glands and in their secretions (Nagler, 2004). Secretory immunoglobulin A (IgA) is the largest immunologic component of saliva. It can neutralize viruses, bacterial, and enzyme toxins and serves as an antibody for bacterial antigens and is able to aggregate bacteria, and inhibiting their adherence to oral tissues (Malamud, 2006). Other immunologic components, such as IgG and IgM, occur in less quantity and probably originate from gingival fluid (Artamonov, 2003).

Among the non-immunologic salivary protein components, there are enzymes (lysozyme, lactoferrin, peroxidase, mucin glycoproteins, agglutinins, histatins, proline-rich proteins, statherins, and cystatins) (Axelsson, 2000).

1.5 Composition of saliva
The water constitutes about 99.4% of unstimulated saliva volume and about 99.5% of stimulated saliva volume, the remaining composed of organic and inorganic constituents, the organic constituents include proteins, carbohydrates and lipids, whereas inorganic constituents include calcium, phosphate, magnesium, sodium, chloride, bicarbonate and hydrogen ions, in addition to traces of zinc, copper, fluoride and strontium (Sevon et al, 2008).

Table (1.1) show the inorganic composition of mixed human saliva.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>1-2</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.2-0.5</td>
</tr>
<tr>
<td>Na$^{+}$</td>
<td>6-26</td>
</tr>
<tr>
<td>K$^{+}$</td>
<td>14-32</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>1-7</td>
</tr>
<tr>
<td>H$_3$PO$_4$ &amp; HPO$_4^{2-}$</td>
<td>2-23</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>17-29</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>2-30</td>
</tr>
<tr>
<td>F$^-$</td>
<td>0.0005-0.005</td>
</tr>
<tr>
<td>SN$^-$</td>
<td>0.1-0.2</td>
</tr>
</tbody>
</table>

(Table 1.1): The average inorganic composition of mixed human saliva in (mM).

1.5.1 Inorganic salivary constituents
Calcium(Ca$^{2+}$)
One of the inorganic components of saliva, about 99% of which is present in skeleton of the humans body while the remaining 1% equally distributed between teeth and soft tissues (FAO, 2004), calcium play an important role in maintaining the integrity of teeth (DeAlmeida et al, 2008). In saliva, calcium present as free ionized and non-ionized bound calcium this depend on the salivary pH in that when the pH is close to the normal the ionized calcium become comprises approximately 50% of the total calcium concentration but when the salivary pH become lower than the normal the concentration of calcium increases and the most of it is present as ionized form (Tenovuo and Lagerlof, 1994; Larsen and Bruun, 1994). The non-ionized calcium is bounded to inorganic phosphate, bicarbonate, proteins as (proline-rich protein, histidine and statherine), acids as (aliphatic acid, citric acid and uric acid) and enzymes as (amylase) in addition to dietary carbohydrates including monosaccharaides, polysaccharides and oligosaccharides (Axelsson, 2000). In saliva the Ca/P ratio were seen to be higher in total females than males (El-Samarrai, 2001).
The concentration of calcium was different between salivary glands in that its concentration is about 2-3 times more in submandibular gland than in parotid gland (Suddick et al., 1980). Different concentrations of calcium in whole human saliva has been demonstrated by many studies as shown in Table (1.2).

**Table (1.2): Calcium ions concentration in human whole saliva.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age</th>
<th>Type of saliva</th>
<th>Results (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon and Feller</td>
<td>U.S.A</td>
<td>1979</td>
<td>11-16</td>
<td>Unstimulated</td>
<td>43±16.3</td>
</tr>
<tr>
<td>Lagerlof and Lidqvst</td>
<td>Sweden</td>
<td>1982</td>
<td>19-49</td>
<td>Stimulated</td>
<td>24.4</td>
</tr>
<tr>
<td>Kullaam Mikkonen</td>
<td>Finland</td>
<td>1985</td>
<td>20-23</td>
<td>Unstimulated</td>
<td>180±120</td>
</tr>
<tr>
<td>Obry</td>
<td>France</td>
<td>1987</td>
<td>7-16</td>
<td>Unstimulated</td>
<td>1.21±0.38</td>
</tr>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>0.77±0.13</td>
</tr>
<tr>
<td>Sulaiman</td>
<td>Iraq</td>
<td>2000</td>
<td>20-32</td>
<td>Stimulated</td>
<td>1.28±1.63</td>
</tr>
<tr>
<td>El-Samarrai</td>
<td>Iraq</td>
<td>2001</td>
<td>11-16</td>
<td>Stimulated</td>
<td>0.41±0.44</td>
</tr>
<tr>
<td>Al-Nowaiser</td>
<td>London</td>
<td>2003</td>
<td>4-13</td>
<td>Stimulated</td>
<td>60.12±11.8</td>
</tr>
<tr>
<td>Al-Yasari</td>
<td>Iraq</td>
<td>2006</td>
<td>33-55</td>
<td>Stimulated</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>Al-Safi</td>
<td>Iraq</td>
<td>2007</td>
<td>17-66</td>
<td>Unstimulated</td>
<td>1.09±0.05</td>
</tr>
<tr>
<td>Daoud</td>
<td>Iraq</td>
<td>2008</td>
<td>15-75</td>
<td>Unstimulated</td>
<td>0.48±0.25</td>
</tr>
<tr>
<td>Al-Rubbaey</td>
<td>Iraq</td>
<td>2009</td>
<td>20-25</td>
<td>Stimulated</td>
<td>1.68±0.24</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-50</td>
<td>Stimulated</td>
<td>1.77±0.22</td>
</tr>
</tbody>
</table>
| Al-Jorrani           | Iraq     | 2012 | 13-15| Stimulated     | 80.60±22.97      

**Inorganic phosphorus (P)**

Inorganic phosphorus is the second abundant inorganic element in skeleton of human body after calcium, in that 85% of which is present in skeleton, in nature its usually found to be combined with oxygen as phosphate (Rockville, 2005). Its concentration varies between salivary glands in that its higher in parotid than submandibular gland, while its concentration is very low in minor salivary gland secretion (Dawes and Whelten, 1996). Saliva consist of phosphoric acid (H₃PO₄) and primary (H₂PO₄⁻), secondary (HPO₄²⁻) and tertiary (PO₄³⁻) inorganic phosphate ions, all of these ions comprise about 80% of the salivary inorganic phosphate (Bodgen and Klevay, 2000). About 10-20% of the salivary inorganic phosphate presents in complex with other ions as calcium and also as organic compounds like phosphorylated carbohydrates, phospholipid, lipoprotein, etc (Nordin, 2002).
There is an inverse association between inorganic phosphate concentration and salivary flow rate in that the total inorganic phosphate concentration falls with an increasing salivary flow rate and this might result in under saturation of saliva with respect to tooth minerals (Harris and Godoy, 2004). As the salivary flow rate increases, so does the pH of the saliva, this increase in saliva pH alters the proportion of the four phosphate species, in such that there is fall in the level of the primary ion, but a dramatic increase in the concentration of the tertiary ion (PO$_4^{3-}$) which is the important ionic species with respect to the solubility of tooth mineral, thus the higher the flow rate, the higher the calcium and tertiary phosphate ions, and the more effective saliva in reducing demineralization and promoting remineralization of the teeth (Axelsson, 2000). Different concentrations of salivary inorganic phosphorus in whole saliva has been recorded by different studies as shown in Table (1.3).

### Table (1.3): Phosphorus concentrations in human whole saliva.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age</th>
<th>Type of saliva</th>
<th>Results (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon and Feller</td>
<td>U.S.A</td>
<td>1979</td>
<td>11-16</td>
<td>Unstimulated</td>
<td>43±16.3</td>
</tr>
<tr>
<td>Shaw</td>
<td>U.K</td>
<td>1983</td>
<td>13-15</td>
<td>Unstimulated</td>
<td>90-117 (Range)</td>
</tr>
<tr>
<td>Ben-Aryeh</td>
<td>Palestine</td>
<td>1984</td>
<td>20-60</td>
<td>Unstimulated</td>
<td>201.3±37.16</td>
</tr>
<tr>
<td>Kullaa-Mikkonen</td>
<td>Finland</td>
<td>1985</td>
<td>20-32</td>
<td>Unstimulated</td>
<td>39.33±9.91</td>
</tr>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>3.81± 1.06</td>
</tr>
<tr>
<td>Sulaiman</td>
<td>Iraq</td>
<td>2000</td>
<td>20-32</td>
<td>Stimulated</td>
<td>4.43±5.05</td>
</tr>
<tr>
<td>Al-Yasari</td>
<td>Iraq</td>
<td>2006</td>
<td>33-55</td>
<td>Stimulated</td>
<td>2.98 ±0.17</td>
</tr>
<tr>
<td>Jaber</td>
<td>Iraq</td>
<td>2004</td>
<td>25</td>
<td>Unstimulated</td>
<td>2.44±0.93</td>
</tr>
<tr>
<td>Al-Safi</td>
<td>Iraq</td>
<td>2007</td>
<td>17-66</td>
<td>Unstimulated</td>
<td>4.36 ±0.19</td>
</tr>
<tr>
<td>Daoud</td>
<td>Iraq</td>
<td>2008</td>
<td>15-75</td>
<td>Unstimulated</td>
<td>2.50±0.90</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-50</td>
<td>Stimulated</td>
<td>3.00±0.65</td>
</tr>
<tr>
<td>Al-Jorrani</td>
<td>Iraq</td>
<td>2012</td>
<td>13-15</td>
<td>Stimulated</td>
<td>84.62±01.72</td>
</tr>
</tbody>
</table>

**Magnesium (Mg$^{2+}$)**

In mixed saliva magnesium concentration was possibly 0.1-0.7 mg % but it could vary according to technique available (Cutress, 1983; Agha-Hossein, 2006). Studies showed much controversy about the relation of Mg to dental caries (Mass et al, 2002; Al-Zaidi, 2007). The influence of Mg on acid resistance of hard dental tissues remains unclear. There is much controversy regarding the influence of Mg on biological processes, undoubtedly, Mg modifies the activity of alkaline phosphatase which participates in the formation of physiological hydroxyapatite crystals and this could increase or decrease susceptibility of enamel to dissolution (Waszkiel et al, 2004). Imbalances in magnesium metabolism are common and are associated with different pathological conditions, studies suggested that periodontitis may be a risk factor for cardiovascular diseases, which have also been associated with Mg deficiencies (Scannapieco et al, 2003; Meisel et al, 2005). Thus, reduced Mg concentrations are associated with enhanced inflammatory response to bacterial challenge (Malpuech-Brugere et al, 2000).

Different concentrations of salivary magnesium in whole saliva has been recorded by different studies as shown in Table (1.4).
Table (1.4): Magnesium concentration in human whole saliva.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age</th>
<th>Type of saliva</th>
<th>Results (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>2.31 ± 0.68</td>
</tr>
<tr>
<td>Kashmoola</td>
<td>Iraq</td>
<td>2000</td>
<td>14-88</td>
<td>Unstimulated</td>
<td>5.579±0.776</td>
</tr>
<tr>
<td>Al-Rawi</td>
<td>Iraq</td>
<td>2001</td>
<td>20-30</td>
<td>Unstimulated</td>
<td>9.46 ± 2.07</td>
</tr>
<tr>
<td>Abdullah</td>
<td>Iraq</td>
<td>2002</td>
<td>25-49</td>
<td>Unstimulated</td>
<td>0.22 ± 0.16</td>
</tr>
<tr>
<td>Al-Zaidi</td>
<td>Iraq</td>
<td>2007</td>
<td>20-24</td>
<td>Stimulated</td>
<td>0.80 ± 0.14</td>
</tr>
<tr>
<td>Al-Jobouri</td>
<td>Iraq</td>
<td>2007</td>
<td>16-18</td>
<td>Stimulated</td>
<td>0.98 ± 0.58</td>
</tr>
<tr>
<td>Al-Bahadli</td>
<td>Iraq</td>
<td>2007</td>
<td>22-52</td>
<td>Stimulated</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Ghulam</td>
<td>Iraq</td>
<td>2007</td>
<td>20-25</td>
<td>Stimulated</td>
<td>0.40 ± 0.24</td>
</tr>
<tr>
<td>Yas</td>
<td>Iraq</td>
<td>2009</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>0.18 ± 0.33</td>
</tr>
<tr>
<td>Yas</td>
<td>Iraq</td>
<td>2009</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>0.22 ± 0.43</td>
</tr>
</tbody>
</table>

**Zinc (Zn^{2+})**

Concentrations of Zn in human mixed saliva vary greatly, ranging from 880-1350 ppm (Freeland-Graves et al, 1981). Studies showed that impaired taste acuity is a typical feature of Zn deficiency which is may be related to its role in the integrity of oral epithelium and taste buds (Watanabe et al, 2005). Studies reported a significant role of zinc ion in relation to increase resistance of teeth to dental caries. Its presence in saliva may enhance remineralization (El-Samarrai, 2001; Al-Zaidi, 2007). It was found that the Zn/Cu molar ratios in whole saliva were significantly decreased in subjects with more than three decayed teeth compared with those with no caries (Borella et al, 1994). It was postulated that Zn deficiency is a potential risk factor for oral and periodontal diseases by decreasing the lymphocyte concentration and depressing the T and B lymphocyte function (Zaichk and Bagirov, 1994; Shankar and Prasad, 1998; Orbak et al, 2007). The level of Zn in saliva is not affected by flow rate, it shows circadian variations peaks at the late morning (Dreizen et al, 1970). Results suggest that mixed saliva zinc is not a useful index of zinc status; however, salivary sediment zinc may be a sensitive parameter if contamination can be avoided (Freeland-Graves et al, 1981).

Different concentrations of salivary zinc in whole saliva has been recorded by different studies as shown in Table (1.5).
Table (1.5): Zinc concentration in human whole saliva.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Type of saliva</th>
<th>Results(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henkin</td>
<td>U.S.A</td>
<td>1975</td>
<td>Stimulated</td>
<td>0.05 ± 0.003</td>
</tr>
<tr>
<td>Free land-Graves</td>
<td>U.S.A</td>
<td>1981</td>
<td>Stimulated</td>
<td>2.66 ± 0.89</td>
</tr>
<tr>
<td>Harrap</td>
<td>U.S.A</td>
<td>1984</td>
<td>Stimulated</td>
<td>2.43</td>
</tr>
<tr>
<td>Ibrahim</td>
<td>Iraq</td>
<td>1993</td>
<td>Stimulated</td>
<td>0.04-0.07(Range)</td>
</tr>
<tr>
<td>Kashmoola</td>
<td>Iraq</td>
<td>2000</td>
<td>Unstimulated</td>
<td>0.43±0.061</td>
</tr>
<tr>
<td>Al-Rawi</td>
<td>Iraq</td>
<td>2001</td>
<td>Unstimulated</td>
<td>0.19±0.04</td>
</tr>
<tr>
<td>El-Samarrai</td>
<td>Iraq</td>
<td>2001</td>
<td>Stimulated</td>
<td>0.130±0.170</td>
</tr>
<tr>
<td>Abdullah</td>
<td>Iraq</td>
<td>2002</td>
<td>Unstimulated</td>
<td>0.207±0.04</td>
</tr>
</tbody>
</table>

**Sodium(Na⁺)**

Sodium is one of the major extracellular ions that represent the principle electrolytes of human body fluids (Sheng, 2000). It plays an important role in maintenance of normal distribution of water and osmotic pressure (Scott et al, 1999). There is a direct association between the sodium concentration in saliva and salivary flow rate in that sodium concentration in saliva increase by increasing salivary flow rate (Harris and Godoy, 2004). The secretion of saliva involves two major steps primarily an acinar secretion yielding a primary saliva which has an ionic composition similar to that of plasma and secondarily when the electrolyte excretion and reabsorption in the duct system occur here the primary saliva is modified that mean the sodium is actively reabsorbed and the potassium is secreted but with a slower rate, so during maximum salivation, there will be no much time for modification process to occur therefore, sodium concentration in saliva increase while potassium concentration decreased (Tuner and George, 1988; 1988; Guyton and Hall, 2000; Johnson, 2003). Some studies were done to measure the sodium level in human whole saliva as shown in (Table 1.6).

Table (1.6): Sodium ions concentration in human whole saliva.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Type of saliva</th>
<th>Results(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogen</td>
<td>U.S.A</td>
<td>1971</td>
<td>Stimulated</td>
<td>19.0-35.5(Range)</td>
</tr>
<tr>
<td>Ben-Aryeh</td>
<td>Palestine</td>
<td>1984</td>
<td>Unstimulated</td>
<td>184.6 ± 8.88</td>
</tr>
<tr>
<td>Kullaa-Mikkonen</td>
<td>Finland</td>
<td>1985</td>
<td>Unstimulated</td>
<td>902.9 ± 207.9</td>
</tr>
<tr>
<td>Al-Milli</td>
<td>Iraq</td>
<td>1995</td>
<td>Unstimulated</td>
<td>18.59 ± 4.65</td>
</tr>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>Unstimulated</td>
<td>11.91 ± 3.60</td>
</tr>
<tr>
<td>El-Samarrai</td>
<td>Iraq</td>
<td>2001</td>
<td>Stimulated</td>
<td>10.06 ± 4.52</td>
</tr>
<tr>
<td>Radhi</td>
<td>Iraq</td>
<td>2009</td>
<td>Stimulated</td>
<td>13.85 ± 3.03</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>Stimulated</td>
<td>7.97 ± 2.97</td>
</tr>
</tbody>
</table>
**Potassium (K⁺)**

It is one of the abundant minerals in the human body, about 98% of potassium in the body is found inside the body cells while the remaining about 2-3% found outside the body cells, so it is considered to be the major intracellular ion and its concentration in saliva is higher than its concentration in plasma (Benyon, 1998; Halperin and Kamel, 1998). Potassium plays an important role in cell function by regulating the osmotic pressure and neuromuscular transmission and also participates in the synthesis of protein and glycogen (Rose, 1984). There was a controversy noticed about the potassium concentration in saliva and the rate of salivary secretion in that some reported a negative correlation between salivary potassium concentration and salivary secretion rate while the other showed that the potassium concentration remain more or less constant regardless of the rate of salivary secretion (Al-Jaafery, 1999; El-Samarrai, 2001; Kanform and Lamster, 2002; Johnson, 2003). Some studies that measures the potassium level in human whole saliva are shown in Table (1.7).

### Table (1.7): Potassium ion concentration in human whole saliva.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age (year)</th>
<th>Type of saliva</th>
<th>Results (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogen</td>
<td>U.S.A</td>
<td>1971</td>
<td>18-45</td>
<td>Stimulated</td>
<td>13.9-22.5 (Range)</td>
</tr>
<tr>
<td>Ben-Aryeh</td>
<td>Palestine</td>
<td>1984</td>
<td>20-60</td>
<td>Unstimulated</td>
<td>478.4 ± 95.4</td>
</tr>
<tr>
<td>Kullaa-Mikkonen</td>
<td>Finland</td>
<td>1985</td>
<td>20-32</td>
<td>Unstimulated</td>
<td>113.6 ± 59.1</td>
</tr>
<tr>
<td>Al-Milli</td>
<td>Iraq</td>
<td>1995</td>
<td>24-68</td>
<td>Unstimulated</td>
<td>23.30 ± 5.53</td>
</tr>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>21.00 ± 5.10</td>
</tr>
<tr>
<td>El-Samarrai</td>
<td>Iraq</td>
<td>2001</td>
<td>11-16</td>
<td>Stimulated</td>
<td>19.43 ± 3.75</td>
</tr>
<tr>
<td>Norri</td>
<td>Iraq</td>
<td>2004</td>
<td>6-11</td>
<td>Unstimulated</td>
<td>28.98 ± 5.70</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-50</td>
<td>Stimulated</td>
<td>8.63 ± 1.25</td>
</tr>
</tbody>
</table>

Studies showed that the saliva also containing a trace elements such as chromium, iron, nickel and aluminum as shown in Table (1.8), and these elements may incorporate the outer enamel surface during demineralization and remineralization processes and thus changing the tooth resistance against dental caries (Jane and Victoria, 2011). A limited studies showed that there was a controversy about the relation between these trace elements and dental caries in that, Koji et al (2011) showed that there was a positive correlation between iron and aluminum in saliva and dental caries, where as Belskaya and Golovanova (2008) showed that there was a negative correlation between these elements and dental caries.
1.5.2 Organic salivary constituent

Lipids are compounds that are soluble in organic solvents and insoluble in water, they are chemically either compounds that give us fatty acids on hydrolysis or complex alcohols that combine with fatty acid to form esters (Rifai and Warnick, 2006). In human body the lipids consist of total cholesterol, triglyceride (TG) and phospholipids (Darlene et al, 1998; Insel and Paul, 2003; Smith et al, 2003).

Total cholesterol is a steroid and considered to be a precursor of many steroids that are of physiological importance like bile acids and steroid hormones (Stipanuk, 2000). It is exerted either as a cholesterol in bile or converted to bile acid and then excreted (Smith et al, 2003). It is fat like substance that does not mix with blood, so it combines with protein to form lipoproteins (Insel and Paul, 2003). Cholesterol play an important role in human body in repairing cell membrane, manufacture of vitamin D on skin surface, producing hormones such as testosterone and estrogen and helping cells in the brain that is important for memory (Gillis, 2007), cholesterol measurement become of great importance after recognizing its strong association with cardiovascular diseases (Warnick and Ramaley, 2001). Triglyceride is a fatty acid esters of glycerol that are transported from the intestine and liver to various tissues (Zilva et al, 1994; Smith et al, 2003).
Low density lipoprotein is a combination of a cholesterol and protein that circulate through the body and carry cholesterol from the liver and small intestine to other tissues and cells in the body that need it. High level of low density lipoprotein associated with an increased risk of atherosclerosis and coronary heart disease, so it is also called bad cholesterol (Castellietal, 1997; Jones, 2004).

High density lipoprotein are also lipoprotein, in contrast to low density lipoprotein it seem to protect the vascular walls of plaque, high level of high density lipoprotein therefore seems to prevent atherosclerosis (Sharp et al, 2000; Peter, 2005). Very low density lipoprotein contains the highest amount of triglyceride in the circulation come in contact with lipoprotein lipase which remove triglyceride from very low density lipoprotein for storage or energy production (Huagy et al, 1998; Insel and Paul, 2003).

Salivary lipids are mostly glandular in origin, but some believed that at least some of the lipid in the oral cavity is of sebaceous origin (Haley and Wertz, 2007). About (80-100 mg/L) of lipids present in the major salivary gland secretion, about 75% of lipids in these secretion are neutral lipids, (20-30%) glycolipids and about (2-5%) phospholipids. Neutral lipids are mainly free fatty acids, cholesterol and monoglycerides, diglycerides and triglycerides, whereas minor salivary glands contain even more lipid about (400 mg/L) in which glycolipid forming the major group (Tenovuo and Lagerlof, 1994). In the parotid and sublingual glands a high fat diet cause lipid accumulation but without any alterations while submandibular gland show a partial resistance to intracellular lipid accumulation (Pisiriciler et al, 2009). It was found that salivary lipids of healthy adults reflect serum lipids concentration to some extent (Karajalainen et al, 1997; Lu et al, 1999; Tabak, 2001; Warnick and Ramaley, 2001; Lawrence, 2002).

A number of studies were done to show the lipid profile concentration in human whole saliva as shown in Table (1.9).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age year</th>
<th>Type of saliva</th>
<th>Results(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karajalinen</td>
<td>Finland</td>
<td>1997</td>
<td>34-39</td>
<td>Stimulated</td>
<td>Total cholesterol 0.02-5.46</td>
</tr>
<tr>
<td>Mattila et al</td>
<td>Finland</td>
<td>2000</td>
<td>85</td>
<td>Stimulated</td>
<td>Total cholesterol 6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HDL 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T.G 1.7</td>
</tr>
<tr>
<td>Al-Rawi and Atiyah</td>
<td>Iraq</td>
<td>2008</td>
<td>40-50</td>
<td>Unstimulated</td>
<td>Total cholesterol 0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HDL 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T.G 0.34</td>
</tr>
<tr>
<td>Al-Rubbaey</td>
<td>Iraq</td>
<td>2009</td>
<td>20-25</td>
<td>Stimulated</td>
<td>Total cholesterol 0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HDL 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T.G 0.21</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-50</td>
<td>Stimulated</td>
<td>Total cholesterol 0.183</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HDL 0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T.G 0.045</td>
</tr>
</tbody>
</table>
**Total proteins** are a group of complex organic macromolecules that contain carbon, hydrogen, oxygen, nitrogen and usually sulfur and are composed of one or more chains of amino acid linked by peptide bonds and are folded into a specific three dimensional shape maintained by further chemical bonding (Maiti, 1995; Gronder et al, 2000). Proteins are essential components of all living cells, consisting of many substances that play an important role in proper functioning of the body cells, tissues and organs (Bennick, 2007). The proteins can be divided into two general classes these are functional proteins and structural proteins, the functional proteins include enzymes, carrier proteins, storage proteins, antibodies and certain hormones, while structural proteins help to organize the structure of tissues and organs and give them strength and flexibility (Groff and Gropper, 2000).

In saliva the concentration of total protein is less than its concentration in plasma in that total protein concentration in saliva is only about one-thirtieth of its concentration in plasma (Dawes, 1996). Despite of its low concentration in salivary glands secretion (0.15-0.64 g/L) but there are more than 40 proteins have been identified in saliva (Mandel, 1987). Proline-rich protein constitutes about 65% of total salivary protein and about 25% of total protein concentration being α-amylase, lactoferrin, histatins, statherins, cystatins, immunoglobulin, mucins, tissue growth factors, etc all these salivary proteins have a remarkable diverse range of functions (Rudney, 1995; Hay and Bowen, 1996; Amerongen et al 2007; Bennick, 2007). Among these proteins, histatins and statherin play an important role in controlling the level of calcium and phosphate in saliva and preventing their fall-out and thus maintaining super saturation in relation to hydroxyapatite, also they prevent a rapid drop in salivary pH and aid in its quicker return back to the resting level, in addition they both are antifungal and help to prevent mucosal infection (Harris and Godoy, 2004). The most important salivary proteins that have antimicrobial properties are lysozyme, lactoferrin, salivary peroxidase and secretary IgA, the lactoferrin combine with iron and copper to deprive bacteria of these essential elements, while salivary peroxidase react with the saliva to form the antimicrobial compound hypothiocyanate which in turn inhibit the capacity of the bacteria to fully use glucose (Oho et al, 2002; Harris and Godoy, 2004; Amerongen et al, 2007).

In the oral cavity the main access for phagocytic cells and their antibacterial products is through the gingival crevice and the tonsils (Harris and Godoy, 2004). Different concentrations of the total protein in whole saliva have been demonstrated by many studies as shown in Table (1.10).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age(year)</th>
<th>Type of Saliva</th>
<th>Results(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Jaafery</td>
<td>Iraq</td>
<td>1999</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>1.418± 0.39</td>
</tr>
<tr>
<td>Abass</td>
<td>Iraq</td>
<td>2006</td>
<td>35-45</td>
<td>Unstimulated</td>
<td>1.218± 0.526</td>
</tr>
<tr>
<td>Yas</td>
<td>Iraq</td>
<td>2009</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>5.415± 4.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55-65</td>
<td>Unstimulated</td>
<td>3.8 ± 2.911</td>
</tr>
<tr>
<td>Al-Rubbaey</td>
<td>Iraq</td>
<td>2009</td>
<td>20-25</td>
<td>Unstimulated</td>
<td>22.29± 5.86</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-50</td>
<td>Stimulated</td>
<td>0.32± 0.15</td>
</tr>
</tbody>
</table>

Table (1.10): Summary of studies concerning the concentration of total protein in saliva.
Salivary enzymes

Saliva containing a variable concentrations of many enzymes that can be produced by salivary glands, oral microorganisms, polymorphonuclear leukocytes and oral epithelial cells derived from gingival crevicular fluid and the most important of these enzymes are lysozyme, lactoferrin, peroxidase, mucin glycoproteins, agglutinins, histatins, proline-rich proteins, statherins, and cystatins, these enzymes will be discussed later in oral immune system, also containing another enzymes such as amylase, acid phosphatase, lipase and esterase (Axelsson, 2000).

Amylase play an important role in assisting digestion as discussed before in digestion as a function of saliva (Grigoriev and Nikolaeva, 2003).

Acid phosphatase and esterase are an important enzymes that can be used as a markers for monitoring periodontal diseases in that, Streckfus and Bigler (2002) showed a positive correlation between the level of salivary acid phosphatase and calculus formation which in turn increase the periodontal diseases, they found that saliva of adult periodontitis patients revealed a highest enzyme activities with acid phosphatase than that of healthy individuals who revealed lowest enzyme activity.

Another study showed a positive correlation between salivary esterase and calculus formation, it was found that the esterase activity of whole saliva was higher in individuals with periodontal disease than in periodontally healthy subjects (Taba and Kinney, 2005).

Lipase plays an important role in fat digestion in newborn infants as their pancreatic lipase still needs some time to develop, it also has a protective function, helping to prevent bacterial build-up on the teeth and washing away adhered food particles (Tenovuo, 2002).

Cells in saliva

In addition to desquamated epithelial cells, the saliva contains all forms of leukocytes, of which the principal cells are polymorphonuclear leukocytes, and the number of polymorphonuclear leukocytes varies from person to person at different times of the day and increase in gingivitis (Edgar and Dawes, 2004). Polymorphonuclear leukocytes reach the oral cavity by migrating through the lining of the gingival sulcus by gingival crevicular fluid, living of polymorphonuclear leukocytes in saliva are sometimes referred to as orogranulocytes, and their rate of migration into the oral cavity is termed the orogranulocytic migratory rate. Some investigators think that the rate of migration is correlated with the severity of gingival inflammation and is therefore a reliable index for assessing gingivitis (Chiappin and Antonelli, 2007).

Glucose in saliva

In comparison of healthy person with stimulated saliva to another healthy person with unstimulated saliva, there is an increase in salivary flow rate and decrease in salivary glucose concentration, but the glucose excretion remain unchanged in case of stimulated saliva. On the other hand in diabetic patient there is an increase in salivary flow rate, while the salivary glucose concentration and glucose excretion remain unchanged under the same experimental conditions as shown in Table (1.11) (Jurysta and Bulur, 2009).

Salivary glucose concentration and glucose excretion were appear to be much higher in diabetic patient than in healthy subject for both stimulated and unstimulated saliva (Al-Hayali et al., 2004).

In diabetic patient with poor glycemic control there was a controversy about the correlation between blood glucose level and salivary glucose level, in that some reported that there is a weak correlation ( Mata, Marques, and Rocha, 2004), others showed that there was no correlation between salivary glucose level and blood glucose level (Ben-Aryeh et al, 1988), where as Tenovuo et al. (1986) analyzed glucose levels in more than one hundred simultaneously taken stimulated whole saliva samples and blood samples of seven patients, the variations in salivary glucose were found to be extensive, and the correlation between salivary and blood glucose was highly individual in that, some subjects showed high correlations, while some others showed no change in salivary glucose, even when their blood glucose levels were very high.

Glucose in parotid saliva is more strongly related to blood glucose levels than glucose in mixed saliva (Ben-Aryeh et al, 1988) or in gingival crevicular fluid (Kjellman, 1970; Ficara et al, 1975). Andersson et al. (1998) reported that, after a standardized carbohydrate load, glucose levels in parotid saliva increased even in healthy subjects.
Table (1.11): Unstimulated and stimulated data in normal and diabetic subjects.

<table>
<thead>
<tr>
<th>Type of saliva</th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose concentration (mM)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>78.7 ± 9.2</td>
<td>201.9 ± 34.9</td>
</tr>
<tr>
<td>Stimulated</td>
<td>29.7 ± 8.1</td>
<td>203.6 ± 37.2</td>
</tr>
<tr>
<td><strong>Salivary flow (mL/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>0.82 ± 0.17</td>
<td>1.53 ± 0.13</td>
</tr>
<tr>
<td>Stimulated</td>
<td>2.17 ± 0.14</td>
<td>1.63 ± 0.10</td>
</tr>
<tr>
<td><strong>Glucose excretion (nmol/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>60.9 ± 7.1</td>
<td>60.9 ± 7.1</td>
</tr>
<tr>
<td>Stimulated</td>
<td>59.8 ± 16.5</td>
<td>59.8 ± 16.5</td>
</tr>
</tbody>
</table>

(Jurysta, 2009)

**Bacteria in saliva**

The average of salivary pH was 6.7 which vary according to the salivary flow rate and this average favours the growth of many microbes, but a few specific species of these microbes are believed to affect the oral health, among these bacteria are oral streptococci that can be divided into four groups these are; **Mitis group** includes *S.mitis*, *S.sanguis*, *S.parasanguis*, *S.gordoni*, *S.crista*, *S.oralis* together with *S.pneumoniae*, all of them are found in mature dental plaque, and was reported to play an essential role in gingivitis and periodontitis, also were isolated from carious dentin and infected root canal (Murray et al, 1999), **Anginosus group** includes *S.anginosus*, *S.constellatus*, *S.intermedius*; they are clinically significant, being associated with purulent infections at oral and non-oral sites (Samaranayake et al, 2002), **Salivarius group** includes *S.salivarius*, *S.vestibularis* and *S.thermophiles*, of these *S.salivarius* has been found to be able to produce caries like lesion in vitro but there were some evidence that there was weak relationship between these organisms and dental caries in vivo (Murray et al., 1999), and **Mutans group** includes *S.mutans*, *S.sobrinus*, *S.cricetus*, *S.rattus*, *S.downei*, and *S.macacae*. These species are associated with dental caries in human and animals (Samaranayake et al, 2002).

Other species found in saliva are *Lactobacilli* (*Lactobacillus acidophilus*) and *Actinomyces viscosus*, that are most closely associated with dental caries in particular root caries (Neville and Damm, 2009).

*Streptococcus mutans* and *Lactobacilli* are considered acidogenic bacteria because they have the ability to produce lactic acid from sugar, also they are considered aciduric bacteria because they have the ability to tolerate acidic environments, on the other hand *Actinomyces viscosus* is associated with root caries but its role is not clear (Cotter and Hill, 2003).

**1.6 Salivary flow rate**

The normal salivary flow rate are approximately (0.25-0.35 ml/min) for unstimulated saliva and (1-3 ml/min) for the stimulated saliva (Dawes, 2004; McDonald et al, 2004). Both rates stimulated and unstimulated shows a wide range as shown in Table (1.12) which demonstrates the studies concerning salivary flow rate among different age groups in different population.

Unstimulated (resting) saliva is a mixture of secretions which enter the mouth in the absence of exogenous stimuli (Amerongen et al, 2007; Stooky, 2008). Several factors affecting unstimulated salivary flow rate as the degree of hydration, body position, exposure to light, previous stimulation, circadian rhythms (peak during late afternoon), circadian rhythms (peak during winter), medications (Leone and Oppenheim, 2001; Thie et al, 2002; Guggenheimer, 2003).
Stimulated saliva is secreted in response to either masticatory or gustatory stimulation and to a lesser extent by activation of the vomiting center (Dawes, 1996; Ghezzi et al, 2000; Stooky, 2008). Stimulated salivary flow rate is affected by nature of the stimulus, vomiting, smoking, gland size, gag reflex, olfaction and food intake (Sreebny, 2000; Chausau et al, 2002; Inoue et al, 2009).

The salivary flow rate was found by many studies to be increase with age (El-Samarrai, 2001; Tulunoglu et al, 2006; Al-Joubori, 2007). It has been found that aging is associated with diminished numbers of acinar cells (secretory units) which is of special importance because acinar cells are the only cell type within salivary glands capable of transporting fluids (Drummond et al, 1995); in addition to an increase in fibrous and fatty tissues within salivary glands (Steele and Walls, 2003).

Males had higher flow rates than females, probably due to the larger salivary gland size in men (Sawair et al, 2009; Guy, 2012). Many studies showed that females had significantly lower flow rates than males for both unstimulated (resting) whole saliva and stimulated saliva, in addition women had lower buccal and labial saliva secretion rates than men (Fure and Zickert, 1990; Percival et al, 1994; Eliasson et al, 2006), the same result had been found by El-Samarrai (2001), Tulunoglu et al (2006), Al-Dafaai (2007), Yas (2009) while Heft and Baum(1981), Fischer and Ship (1999), Al-Joubori (2007) found that there is no difference in flow rate between the two genders.

Many different studies were made to show the salivary flow rate in different countries as shown in Table (1.12).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Origin</th>
<th>Year</th>
<th>Age</th>
<th>Type of saliva</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khalaf</td>
<td>Iraq</td>
<td>1994</td>
<td>11-25</td>
<td>Unstimulated</td>
<td>0.64 ± 0.40</td>
</tr>
<tr>
<td>Sulaiman</td>
<td>Iraq</td>
<td>2000</td>
<td>20-32</td>
<td>Stimulated</td>
<td>2.06 ± 2.46</td>
</tr>
<tr>
<td>El-Samarrai</td>
<td>Iraq</td>
<td>2001</td>
<td>11-16</td>
<td>Stimulated</td>
<td>6.83 ± 0.25</td>
</tr>
<tr>
<td>Bayrakter et al</td>
<td>Turkey</td>
<td>2002</td>
<td>21-55</td>
<td>Stimulated</td>
<td>1.52 ± 0.51</td>
</tr>
<tr>
<td>Bayrakter et al</td>
<td>Turkey</td>
<td>2004</td>
<td>18-60</td>
<td>Stimulated</td>
<td>1.64 ± 0.44</td>
</tr>
<tr>
<td>Nederfors et al</td>
<td>Sweden</td>
<td>2007</td>
<td>18-46</td>
<td>Unstimulated</td>
<td>0.62 ± 0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.81 ± 0.68</td>
</tr>
<tr>
<td>Al-Ani</td>
<td>Iraq</td>
<td>2007</td>
<td>11-16</td>
<td>Stimulated</td>
<td>1.19 ± 0.13</td>
</tr>
<tr>
<td>Al-Jobouri</td>
<td>Iraq</td>
<td>2007</td>
<td>16-18</td>
<td>Stimulated</td>
<td>0.81 ± 0.12</td>
</tr>
<tr>
<td>Ghulam</td>
<td>Iraq</td>
<td>2007</td>
<td>20-25</td>
<td>Stimulated</td>
<td>1.28 ± 0.60</td>
</tr>
<tr>
<td>Al-Obaidi</td>
<td>Iraq</td>
<td>2009</td>
<td>22-23</td>
<td>Stimulated</td>
<td>1.22 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unstimulated</td>
<td>0.27 ± 0.16</td>
</tr>
<tr>
<td>Sevon et al</td>
<td>Finland</td>
<td>2008</td>
<td>30-59</td>
<td>Unstimulated</td>
<td>1.52 ± 0.71</td>
</tr>
<tr>
<td>Yas</td>
<td>Iraq</td>
<td>2009</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>0.46 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55-65</td>
<td>Unstimulated</td>
<td>0.38 ± 0.24</td>
</tr>
<tr>
<td>Al-Rubbaey</td>
<td>Iraq</td>
<td>2009</td>
<td>20-25</td>
<td>Stimulated</td>
<td>1.11 ± 0.21</td>
</tr>
<tr>
<td>Masood</td>
<td>Iraq</td>
<td>2010</td>
<td>49-55</td>
<td>Stimulate</td>
<td>1.51 ± 0.81</td>
</tr>
<tr>
<td>Al-Jorrani</td>
<td>Iraq</td>
<td>2012</td>
<td>13-15</td>
<td>Stimulated</td>
<td>1.07 ± 0.35</td>
</tr>
</tbody>
</table>
Xerostomia (dry mouth) can be defined as the subjective sensation of oral dryness that is usually accompanied by reduced salivary volume which in turn leads to drying of oral soft tissues and loss of protective effect of salivary buffers, proteins, and mucins, in addition to changes in the quantitative and qualitative properties of bacterial plaque with increase counts of acidogenic and aciduric bacteria, these consequences can result in significant problems such as dental caries, periodontal diseases, higher risks of candidiasis, and mucositis, which result in an overall reduction in the quality of life, all these considered the main signs of xerostomia (Buhlink et al, 2002; Hjoel et al, 2002; Thomson and Spencer, 2002; Sevon et al, 2008; Younger et al, 2008; Gater, 2008), whereas viscous saliva, sticky saliva, difficulties in talking, difficulties in swallowing, halitosis, altered taste, complaint of dryness, complaint of burning (mouth, lips, or tongue), altered sense of smell, and poorly fitting prostheses are the main symptoms of xerostomia (Urquhart and Fowler, 2006).

Although xerostomia has many causes like medications, autoimmune disease (Sjogren's syndrome and systemic lupus erythematosis), systemic diseases (diabetes, HIV infection, etc) and radiation therapy to patient with head and neck cancer, but about 90% of patients have their xerostomia caused by medication like antihistamine, anti-psychotic, anticholinergic, anticonvulsants, etc (Crossley, 2007).

Simply xerostomia can be managed by performing oral hygiene at least four times daily, after each meal and before bedtimes, use fluoride toothpaste, rinse with a salt and baking soda solution 4 to 6 times daily, avoid citrus juices (oranges, grapefruit, tomatoes), rinse and wipe oral cavity immediately after meals, keep water handy to moisten the mouth at all times, avoid liquids and foods with high sugar content, avoid rinses containing alcohol and salty foods, brush and rinse dentures after meals, apply prescription-strength fluoride get at bedtime as prescribed, use moisturizers regularly on the lips, use of salivary substitutes or artificial saliva preparations (Shirodaria and Kilbourn, 2006; Turner and Ship, 2009).

1.7 Salivary pH

The term pH can be defined as the negative logarithm of the hydrogen ion concentration and it indicates the degree of acidity or alkalinity of a solution, and higher hydrogen ions reduce pH and vice versa. The average of salivary pH is equal to 6.7 and it is vary with salivary flow rate from 5.3 with low flow to 7.8 with peak flow (Tenovuo and Lagerlof, 1994; Bardow et al, 2000; Myers, 2010).

Hydrogen ions secreted via the salivary gland in form of inorganic and organic acids, produced by the oral microbiota and taken by food stuff or acid drinks (Tenovuo and Lagerlof, 1994). The concentration of hydrogen ions in saliva influence most of the chemical reaction occurs in the oral cavity, especially the equilibrium between the calcium and phosphate of the tooth and the surrounding liquid phase (Larsen and Bruun, 1994; Axelsoon, 2000; Humphery and William, 2001).

The critical pH can be defined as the level of pH at which the saliva is exactly saturated with respect to enamel mineral. Below the critical pH enamel may dissolve, calcium and phosphate ions diffuse toward the plaque, whilst above it enamel tends to remineralize (Dawes, 1996; Peter, 2004).

Regulation of oral pH is an important function of salivary buffering systems including; the bicarbonate, phosphate, protein-based buffer system, and urea (Bardow et al, 2000; Peter, 2005). The bicarbonate concentration in buffer system plays a major role in the protection against dental caries and acts mainly to neutralize acids (Anderson and Orchardson, 2003; Peter, 2004).

\[ \text{HCO}_3^- + \text{H}^+ \xrightarrow{\text{Reaction}} \text{H}_2\text{CO}_3 \xrightarrow{\text{Decomposition}} \text{H}_2\text{O} + \text{CO}_2 \]

When an acid is added, bicarbonate release a weak carbonic acid, this rapidly decompose to water and carbon dioxide. Since the mouth is an open system; carbon dioxide will be lost to the atmosphere and this will lead to more carbonic acid production and more carbon dioxide escape from the oral cavity and increase the binding of bicarbonate and hydrogen ions, the end result is the complete removal of the acid (Bradow et al, 2000; Ericsson and Barthal, 2007).

1.8 Viscosity of Saliva

It has been shown that the viscosity of saliva is related to the rate of dental caries, both thick, ropy saliva and thin, watery saliva have been responsible for rampant caries, in that a positive correlation was detected between the viscosity of saliva and the number of decayed, missing, and filled teeth. Patients with thick, ropy saliva invariably had poor oral hygiene and the teeth appear to be covered by stain or plaque, and the rate of dental caries ranged from greater than average to rampant caries (Ralph and McDonald, 2004).
It has been shown that children who consume excessive amounts of carbohydrates often have not only a sparse flow but also a viscous saliva (Walsh, 2006). In some persons the viscosity of saliva also has been shown to increase by consumption of antihistaminic drugs (Hersh, 2002). Although there were limited number of ways to alter the viscosity of saliva, but in some patients the reduction of refined sugar intake may be effective in reducing this viscosity (Pedersen and Bardow, 2002).

1.9 Oral immune system

In general immunity can be defined as the sum of all naturally occurring defense mechanisms that play an important role in human protection against infectious and other diseases, resistance mechanism can be divided into two types these are: non-specific (innate) and specific (acquired) (Hyde, 2000). The nonspecific immunity comprises the cells and mechanisms that defend the host from infection by other organisms, in a non-specific manner, this means that the cells of the innate system recognize, and respond to, pathogens in a generic way, it differs from specific immunity in that it does not confer long-lasting or protective immunity to the host, the cells involved in innate immunity are macrophages, natural killer cells, mast cells in addition to complement system (Bruce et al, 2002).

On the other hand the specific or adaptive immune system is known as an antigen specific and based up on recognition of specific (non-self) antigen throughout process known as antigen presentation, and the cells involved in this type of immunity are special cells of leukocytes called lymphocytes particularly B and T cells, these cells are able to identify the pathogens when the antibodies that lies on their surfaces bind to a specific foreign antigen, in this process immunoglobulin M (IgM) is the first antibody to respond which is soon superseded by immunoglobulin G (IgG) response (Bruce et al, 2002; Rabson et al, 2005). T cells involve helper T cells (CD4+) and cytotoxic T cells (CD8+), helper T cells are considered to be immune response mediators, and play an important role in establishing and maximizing the capabilities of the adaptive immune response, these cells have no cytotoxic or phagocytic activity; and cannot kill infected cells or clear pathogens, but they manage the immune response, by directing other cells to perform these tasks, on the other hand cytotoxic T cells which are also known as killer T cells or cytotoxic T-lymphocyte have the ability to kill virus infected cells or the cells that infected by other pathogens, otherwise they may be damaged or considered to be dysfunctional (Charlis et al, 2001; Antony et al, 2005).

In the oral cavity the soft and hard tissues protected by both non-specific and specific immune factors, the function of these protective factors is to limit the microbial colonization of the oral surfaces and to prevent the penetration of noxious substances through the surfaces and ensuing damage to the underlying tissues (Thylstrup and Fejerskov, 1996; Kidd and Bechal, 1997).

In saliva the non-specific immune factors are represented by lysozyme, lactoperoxidase system, lactoferrin in addition to other salivary components that may act as a bacterial agglutinins. Several of the non-specific immune factors may interact with the specific salivary immune factors which are immunoglobulins, resulting in a mutual amplification of their respective activities (Puy, 2006).

1.9.1 Non-specific immune system

Lysozyme; can hydrolyze the cellular wall of some bacteria, and because it is strongly cationic, it can activate the bacterial “autolysins” which are able to destroy bacterial cell wall components (Amerongen, 2002). Gram-negative bacteria are more resistant to this enzyme due to the protective function of their external lipopolysaccharide layer, other antibacterial mechanisms have been proposed for this enzyme, such as aggregation and inhibition of bacteria adherence (Grigoriev, 2003). Lactoferrin; links to free iron in the saliva causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival such as streptococcus mutans group. Lactoferrin also provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions (Farnaud, 2005).

Peroxidase or sialoperoxidase; offers antimicrobial activity because it serves as a catalyst for the oxidation of the salivary thiocyanate ion by hydrogen peroxide into hypothiocyanate, a potent antibacterial substance, as a result of its consumption, proteins and cells are protected from the toxic and oxidant effects of hydrogen peroxide (Veerman, 2002).
**Proline-rich proteins and statherins;** inhibit the spontaneous precipitation of calcium phosphate salts and the growth of hydroxyapatite crystals on the tooth surface, preventing the formation of salivary and dental calculus (Dam-ante, 2005).

**Cystatins;** are related to acquired film formation and to hydroxyapatite crystal equilibrium and due to its proteinase inhibiting properties, it is surmise they act in controlling proteolytic activity (Blankenvoorde, 1996).

**Histatin;** have antimicrobial activity against some strains of Streptococcus mutans and inhibit hemoagglutination of Porphyromonas gingivalis (Denotti, 2003). These enzymes neutralize the lipopolysaccharides of the external membranes of Gram-negative bacteria and are potent inhibitors of Candida albicans growth and development (Sugiya, 2002).

**Salivary agglutinin;** a highly glycosylated protein frequently associated with other salivary proteins and with secretory IgA, is one of the main salivary components responsible for bacteria agglutination (Amerongen, 2002).

### 1.9.2 Specific immune system

#### 1.9.2.1 Salivary IgA

Salivary glands (major and minor) contain plasma cells which produce IgA, ductal cells of salivary glands transport IgA to saliva. IgA is the main immunoglobulin in saliva although other isotypes are present in a less concentrations that are IgA>IgG>IgM>IgD>IgE (Barr-Agholme et al, 1998). IgA presents in two forms IgA1 and IgA2 in different concentrations according to their presenting sites e.g. in saliva approximately equal concentrations; IgA1 approximately 80-90% in serum, IgA1 about 90% in nasal cavity, IgA2 about 55-60% in lower GIT, salivary IgA usually contains 65-75% IgA1 but with individual variation (Kett et al, 1986).

Difference in biological functions between IgA1 and IgA2 still unclear. IgA1 is protease susceptible which is a group of bacterial enzymes, IgA1 proteases produced by streptococcal species which initiate formation of dental plaque. Proteases holding up antibody function of IgA1 by cleaving it in hinge region of heavy chain (Kilian and Reinholdt, 1986). Lack of 13-peptide sequence in IgA2 make it resistant to cleavage by proteases that exerts its action at this site (Kerr, 1990). Difference in molecular structure between serum IgA and secretary IgA is that; the serum IgA is monomeric like other immunoglobulin classes while secretary IgA is dimeric associated with J-chain and secreting component (sc), this complex known as secretary IgA (s-IgA), it is the product of two cell types: plasma cells produce dimeric IgA with chain then during transport of IgA through secretary epithelial cells secretory component attached (Thylstrup and Fejerskov, 1996).

Figure (1-7) shows a schematic representation of s-IgA.

![Figure (1-7): Schematic representation of s-IgA](Teeuw et al, 2004).
1.9.2.1.1 Secretary IgA and oral diseases (dental caries and periodontal disease)

A local immune protection in the oral and gingival mucosa against pathogens was suggested to be provided by secretary immune system. A higher salivary IgA level was suggested by a higher serum IgA level in some studies (Balaita et al, 1992). Limited Iraqi studies conducted concerning the immunoglobulins levels in saliva of normal individuals are shown in Table (1.13).

Table (1.13): Iraqi Studies Concerning Concentration of Salivary Immunoglobulins A and G in normal individuals.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No. of Samples</th>
<th>Type of Saliva</th>
<th>Age (Years)</th>
<th>Findings (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atiya</td>
<td>1990</td>
<td>48</td>
<td>Unstimulated</td>
<td>18-20</td>
<td>192.10±93.90</td>
</tr>
<tr>
<td>Al-Hijazi</td>
<td>1992</td>
<td>50</td>
<td>Unstimulated</td>
<td>18-48</td>
<td>67.00±39.00</td>
</tr>
<tr>
<td>Kashmoola</td>
<td>2000</td>
<td>45</td>
<td>Unstimulated</td>
<td>14-88</td>
<td>158.00±42.00</td>
</tr>
<tr>
<td>Juma</td>
<td>2003</td>
<td>25</td>
<td>Unstimulated</td>
<td>20-59</td>
<td>159.70±92.70</td>
</tr>
<tr>
<td>Al-Dhaher</td>
<td>2004</td>
<td>12</td>
<td>Unstimulated</td>
<td>6-18</td>
<td>193.50±20.37</td>
</tr>
<tr>
<td>Alrudainy</td>
<td>2006</td>
<td>58</td>
<td>Unstimulated</td>
<td>7-10</td>
<td>70.30±19.50</td>
</tr>
<tr>
<td>Al-Safi</td>
<td>2007</td>
<td>30</td>
<td>Unstimulated</td>
<td>17-66</td>
<td>117.79±3.75</td>
</tr>
<tr>
<td>Al-Zaidi</td>
<td>2007</td>
<td>20</td>
<td>Stimulated</td>
<td>20-24</td>
<td>81.54±38.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kashmoola</td>
<td>2000</td>
<td>45</td>
<td>Unstimulated</td>
<td>14-88</td>
<td>27.00±19.00</td>
</tr>
<tr>
<td>Alrudainy</td>
<td>2006</td>
<td>58</td>
<td>Unstimulated</td>
<td>7-10</td>
<td>31.50±11.90</td>
</tr>
<tr>
<td>Al-Safi</td>
<td>2007</td>
<td>30</td>
<td>Unstimulated</td>
<td>17-66</td>
<td>686.68±74.00</td>
</tr>
</tbody>
</table>

Inhibition of bacterial adherence to dental enamel occur by secreting IgA depending on bacterial strain analyzed, it is related to the tooth surface proven by its presence in the salivary pellicle, secretary IgA bind to surface adhesions as well as neutralize their negative surface charge so it inhibit adherence (Puy, 2006). IgA also bind to mutans streptococci facilitating bacterial aggregation and removal from oral cavity, secretary IgA preventing adverse effect of bacterial toxins and enzymes that secretary IgA is multivalent antibodies (Dowd, 1999).

The increase in antibody levels against streptococcus mutans , either secretary IgA or IgG , can eliminate streptococcus mutans from oral cavity and interfere with its cariogenic activities as shown by studies .some studies shows contradictory results related to the role of secretary IgA in oral cavity (Thylstrup and Fejerskov, 1996; Marcotte and Lavoie, 1998).

A conflicting results of several studies that attempting to correlate a protective effect of IgA against dental caries, technical and interpretive problems appear where determining the effect of a given antibody, establishment of specificity of antibody against bacterial antigen is in need, the IgA may or may not be directed toward cariogenic bacteria, it is problematic to correlate a protective effect of IgA on the temporal progression of dental caries, studies showing that IgA deficiency do not always correlate to increase in caries activity (Dowd, 1999).
A difference between studies that correlate salivary IgA and dental caries some positive, negative, others show no relations (Grahn et al, 1988; Atiya,1990; Al-Zaidi, 2007).

Caries free patients shows a higher salivary IgA levels compared with caries active patients (Benderti et al, 2000). Salivary IgA related to some factors these are: salivary flow rate, age, hormonal factors, smoking, emotional states, physical activities and genetic background (Grundbacher, 1988; Marcotte and Lavoie, 1998).

Secretary IgA antibody do not penetrate the crevice or pocket so it is difficult to see how salivary IgA control subgingival plaque, salivary IgA may control sub gingival plaque formation and composition and its potential for causing disease by modulating the supra gingival plaque accumulation (Wilton et al, 1989).

The role of salivary IgA in the development of gingivitis and periodontitis is unclear. During gingival inflammation a permeability of the crevicular epithelium to IgA increase that causing increase in salivary IgA level (GÜven and Vissher, 1982; Marcotte and Lavoie, 1998).

A higher level of s-IgA observed in parotid saliva in subjects with gingival inflammation. These studies suggest that s-IgA response induced by increase antigenic load from dental plaque (Jalil et al, 1992; Grahn et al, 1998).

Salivary IgA antibodies suspected to periodontopathogens were detected in both healthy and periodontaly diseased subjects, some studies observed no change in the level of s-IgA to Prophyromonas Gingivalis, Actinobacillus Actinomycetemcomitans and B. Ascharolyticus between both groups. Other studies reported an increase in the level of s-IgA to A. Actinomycetemcomitans in some patients with chronic or aggressive periodontitis (Marcotte and Lavoie, 1998).

The majority of the IgA-expressing plasma cells were IgA1, but a greater proportion expressed IgA2 mRNA and J chain mRNA in the gingival tissues (30.5% and 7.5% respectively) than in the periodontal granulation tissues (19% and 0% to 4% respectively). The J-chain or dimeric IgA2-expressing plasma cells were located adjacent to the epithelium, suggesting that this tissue demonstrates features consistent with a mucosal immune response, periodontal epithelium shares features with mucosal epithelium due to the presence of the secretary component in gingival and epithelial cells. The protective effect of secretary IgA might possibly supplanted by a much greater and inadvertently more destructive response (Kinane and Lappin, 2002).

### 1.9.2.2 Salivary IgG

It is the predominant immunoglobulin in blood, lymph, peritoneal fluid and cerebrospinal fluid. In normal adult it constitutes about 75% of total serum immunoglobulins, and in blood it is considered the most abundant antibody produced during secondary humoral immune response (Frazer and Capra, 1999). Human immunoglobulin G can be subdivided into four main types these are IgG1, IgG2, IgG3 and IgG4 and they are numbered according to their decreasing average serum concentration in that the concentration of IgG1 is 60-70% =90 ppm, the concentration of IgG2 is 14-20% = 30 ppm, the concentration of IgG3 is 4-8% =10 ppm and the concentration of IgG4 is 2-6% =5 ppm, these values vary among individuals (Frazer and Capra, 1999; Miyasaki et al, 2002). IgG is the only class of immunoglobulin that can cross the placenta in humans, and it is responsible for protection of the new born during the first months of life, IgG2 is the less efficient complement activator than others (Goldspay et al, 2000).

IgG can perform several functions in that it diffuses more readily than other immunoglobulins into the extravascular spaces where they neutralize bacterial toxins, bind to microorganisms to facilitate their phagocytosis by phagocytic cells, also IgG has the ability to complex with bacteria and consequently activating complement thereby chemotactically attracting leukocytes, histamine release, and lysis of susceptible bacteria. Another function of IgG is opsonization by coating of foreign particles such as bacteria with IgG molecules results in increased phagocytosis by polymorph nuclear leukocytes, which have a binding site on their surfaces specific for parts of the bound IgG (Murray, 1989; Roitt et al, 1998).

### 1.9.2.2.1 IgG and oral diseases (dental caries and periodontal disease).

When the tooth surface are directly exposed to the gingival fluid, the dental plaque is exposed to salivary immunoglobulins, and it may be also exposed to a significant amount of serum immunoglobulins and for this reason the serum immunoglobulin G is believed
to has potential in modulating the oral colonization by plaque-forming bacteria particularly during tooth eruption (Challacombe et al, 1978). A significant proportion of the immunoglobulin molecules, particularly IgG, that become incorporated in dental plaque occurs as fragments as a result of proteolytic degradation by enzymes excreted by plaque bacteria (Camling et al, 1991). Some studies showed that in caries resistant subjects the saliva promote IgG retention significantly more than saliva from caries susceptible subject noted that the presence of active caries lesions may induce the formation of specific IgGs and that they may remain at higher levels for several weeks or months after eradication of the lesions (Lenander-Lumikari and Loimaranta, 2000).

The effects of dental caries on induction of the systemic immune system or cytokine response were studied, and this study suggest a relationship between dental caries and systemic parameter of inflammation, there was an increase in total IgG against Streptococcus Mutans and the concentration of IgG was significantly correlated with DMFT (De Soet et al, 2003). It has been shown that the IgG1 was the most predominant subclass of IgG in uninfected dentinal tubules beneath shallow and deep caries, whereas this subclass was localized on the pulpal end in a non-carious teeth (Hahn and Best, 2006).

Through examination of 126 children with dental caries and 55 children who are caries free (control group), Parkash et al (1994) showed that the salivary IgG was lower among children with dental caries (1600 ± 7.00 ppm) when compared to those who are caries free (3400 ± 29.00 ppm). It has been shown that in 55% of patient with untreated juvenile periodontitis the salivary IgG antibody to A. actinomyctecomitans was significantly elevated, whereas only in 28% of patient treated for juvenile periodontitis the salivary IgG antibody for A. actinomycetemcomitans was elevated, on the other hand 28% of patients with adult periodontitis show a significant elevation in the level of IgG antibody to A. actinomycetemcomitans (Sandholm et al, 2005). Kinane and Lappin (2002) reported that IgG1 mRNA-expressing cells were predominant in the granulation tissues as in the gingival, constituting approximately 65% of the total IgG-expressing plasma cells. Graig et al (2002) noted elevated serum IgG antibody to P. gingivalis which reflects destructive periodontal disease status. Another study of Novak et al (2006) found higher levels of galactose-deficient IgG in sera and gingival crevicular fluid from periodontal disease patients, compared with levels in healthy controls. Furthermore, gingiva from periodontal disease patients exhibited infiltration of IgG-producing plasma cells; many of them contained galactose deficient IgG in the cytoplasm.

1.10 Saliva and dental caries

Dental caries is a transmissible local infectious disease and saliva act as a critical regulator and internal defense system against this disease (Harris and Godoy, 2004; Dawes, 2008).

As mentioned before saliva have many functions such as lubrication, buffering capacity, acting as an ion reservoir that facilitates the remineralization of teeth, and antimicrobial activity all of these functions are largely protective in relation to dental caries (Godoy and Hicks, 2008; Depaola, 2008). Saliva has an important defense function this is probably via providing a constant salivary flow, because it is considered to be transporting system for the buffering agent, the antimicrobial and the mineral content of saliva thus aid in controlling the equilibrium between demineralization and remineralization of tooth structure, also increase salivary flow is essential for diluting acids, flushing food particles embedded around the teeth, clearing refined carbohydrates and physically removing any displaced bacteria (Edgar et al, 2004; Harris and Godoy, 2004).

Salivary flow rate is very important in caries-risk assessment where it is considered a key parameter in this assessment (Dawes, 2008). There is a direct association between salivary flow rate and clearance rate which in turn affecting dental carries formation that means the higher the salivary flow rate, the faster will be the clearance rate and the higher the buffering capacity which will result in a significant reduction in dental carries formation (Hand, 2003; Dawes, 2008).

Several studies were carried out to investigate the relation between salivary flow rate and dental carries, some of these studies found a significant correlation (Sulaiman, 2000; Koseki et al, 2004; Kirkeby, 2006; Younger et al, 2008), while the others found a weak non-significant correlation (El-Sammarai, 2001; Koga-Ito et al, 2004; Waheed, 2006; Faris, 2008; Radhi, 2009; Al-Rubbaey, 2009).
One of the most important functions of saliva is protection of tooth against dissolution which is caused by cariogenic challenge or by dental erosion, this is done by controlling the pH of the oral environment by means of secreted bicarbonate ion and maintaining super saturation state with respect to mineral phase (Dawes, 2008; Stookey, 2008).

At a neutral condition the salivary pH is supersaturated with calcium and phosphate, while when the condition become more acidic the salivary pH will decrease and become more acidic till it reaches to a minimum value which is called critical pH (5.3-5.5) where the saliva are no longer supersaturated with calcium and phosphate, conversely when the salivary pH become more alkaline the saliva become more supersaturated with minerals at this state the calcium-phosphate will be precipitated at the tooth surface not as hydroxyapatite but as calculus (Edger et al, 2004; Gopinath and Arzreeanne, 2006; Nieves and Blue, 2008). Studies that were carried out to investigate the relation between salivary pH level and dental caries showed a controversy in that some studies showed a significant correlation (Sulaiman, 2000; Radhi, 2009), while other studies found a non-significant correlation (El-Samarrai, 2001; Waheed, 2006; Shapira, et al, 2008; Al-Obaidi, 2009; Al-Rubbaey, 2009).

Certain inorganic constituents of saliva such as calcium, phosphate and hydrogen ions in addition to certain trace elements such as fluoride play an important role in respect to the solubility of tooth mineral as shown in Figure (1-8).

The solubility of mineral phase of enamel depend on two factors these are composition of the tooth itself and the pH of the surrounding environment, the composition of teeth is determined during the period of tooth formation some of these minerals may have either positive or negative effects on solubility, for example carbonate and sodium incorporation increase the solubility whereas fluoride reduce it (Chakaborty et al, 2006; Stookey, 2008). The pH will fall to or even below the critical pH after acid production by microorganism so this will initiate demineralization to occur by dissolution of hydroxyapatite crystals to their ionic components and as a result of increased pressure gradient, the calcium and phosphate ions will diffuse from the tooth surface to the surrounding environment, this acid attack can be terminated by depletion of fermentable carbohydrate and by the buffering capacity of both saliva and dental plaque, so that the pH will return back to the resting level and initiating remineralization by precipitation of hydroxyapatite crystals as shown in the following chemical reaction (TenCate, 1996; Stookey, 2008; Godoy and Hicks, 2008).
The relation between salivary calcium and dental caries were investigated by several studies, some of these studies showed a positive correlation in that the concentration of salivary calcium increase with in increasing dental caries (El-Samarrai, 2001; Hussein, 2002; Gandhy and Damle, 2003; Al-Obaidi, 2009), while the other studies found inverse correlation to dental caries (Woltgens et al., 1992; Sulaiman, 2002), where as Karagül et al. (1998), Waheed (2006) and Radhi (2009) found no significant correlation with dental caries.

Several studies were carried out to investigate the relation between the concentration of inorganic phosphate in saliva and dental caries, some of these studies found an inverse correlation in that the concentration of inorganic phosphate in saliva decrease with increasing dental caries (El-Samarrai, 2001; Sulaiman, 2002; Malbotie et al., 2004; Al-Obaidi, 2005), where as some found that the concentration of inorganic phosphate increase with increasing the susceptibility to dental caries (Grandhy and Damle, 2003). On the other hand, other studies showed that there is no relation between the concentration of inorganic phosphate in saliva and dental caries (Dodde et al., 1997; Lenander et al., 1998; Al-Rubbaey, 2009).

Studies concerning relation between the concentration of sodium in saliva and dental caries are equivocal, El-Samarrai (2001) reported a statistically significant positive correlation between the concentration of sodium in saliva and dental caries, while Zahir and Sarkar (2006) reported a significant negative correlation between the concentration of sodium in saliva and dental caries. On the other hand Radhi (2009) found a weak negative correlation with dental caries.

Although the role of salivary potassium in dental caries initiation and progression is not well clear, but some reports showed an inverse relation between the concentration of potassium in saliva and dental caries (El-Samarrai, 2001; Mass et al., 2002; Radhi, 2009).

Studies showed much controversy about the relation of magnesium to dental caries (Mass et al., 2002; Al-Zaidi, 2007). The influence of magnesium on acid resistance of hard dental tissues remains unclear. There is much controversy regarding the influence of magnesium on biological processes, undoubtedly, magnesium modifies the activity of alkaline phosphatase which participates in the formation of physiological hydroxyapatite crystals and this could increase or decrease susceptibility of enamel to dissolution (Waszkiel et al., 2004).

Studies reported a significant role of zinc ion in relation to increase resistance of teeth to dental caries. Its presence in saliva may enhance remineralization (El-Samarrai, 2001; Al-Zaidi, 2007).

Organic components of saliva also play an important role in maintaining equilibrium of oral ecosystem though certain proteins which are essential constituent of the acquired pellicle, encourage bacterial aggregation, are a source of food for certain bacteria and possess an antimicrobial effect because some of them are capable of modifying the bacteria's metabolism and ability to adhere to the surface of the tooth (Puy, 2006).
1.11 Saliva and periodontal disease

Periodontal disease represent periodontal tissue response to the local irritant factors which represented by over growth and differentiation of dental plaque bacteria (Harris and Godoy, 2004). Although one of the most effective antibacterial mechanism of saliva is its washing action (Depaolo, 2008), but there is a little evidence show that the saliva has a direct influence on periodontal disease, this is probably due to inability of saliva to enter the periodontal pocket which contain the periodontal pathogens that are responsible for periodontal disease in addition to net outward flow of gingival crevicular fluid which prevent the entrance of saliva to the periodontal pocket and thus the antibacterial components of saliva loss their ability to affect the bacteria in periodontal pocket (Dawes, 2008). However, in patient with reduced salivary flow rate, the bacterial clearance is reduced and more bacteria will be in the saliva to colonize the oral tissue (Dawes, 2008). Patient who has aggressive periodontitis may has lower resting salivary flow rate and lower concentration of calcium in both resting and stimulated saliva than those without periodontitis (Rahim and Jalil, 2003). The presence of supragingival calculus will provide a favorable site for plaque accumulation this calculus develop predominantly in the lingual surface of mandibular anterior teeth and this is primarily because the sugar clearance is most rapid in this area and the salivary film velocity is highest in the mouth, therefore after consumption of sugar the Stephan curve does not drop and remain shallow and the plaque fluid in this area tend to maintain its super saturation with respect to minerals in calculus, the minerals in calculus underlying the plaque seldom tend to dissolve (Dawes, 2006).

Many studies were carried out to investigate the correlation between periodontal health and inorganic constituents of saliva (calcium and phosphorus) but these studies showed a controversy, in that Sewon et al (1995) found that the calcium present in a high concentration in patient with periodontitis, whereas Nishida et al (2000) showed that high calcium intake related to a more normal alveolar bone, in (2001) El-Samarraei showed that there is no significant association between the level of calcium and phosphorus in saliva and gingival inflammation. Regarding the effect of salivary sodium on periodontal disease, Erdemir and Erdemir (2006) found a negative correlation between salivary sodium and periodontal disease. Some authors found a significant interrelationship between host response mediators (reactive protein, C3 component of complement, interleukin 6) and some enzymes in salivary secretion as measured in saliva and periodontal destruction measured by clinical attachment loss, this finding might help in diagnosis and estimating the course and progression of periodontal destruction, thus providing clinical benefits in treatment planning (Numabe et al, 2004; Aurer et al, 2005; Todorovic et al, 2006).

1.12 Saliva as a biomarker of oral cancer

Molecular markers for the diagnosis of oral cancer can be quested in three levels these includes;

level 1 changes in the cellular DNA: Typical changes in the host DNA of dysplastic or cancer cells include point mutations, deletions, translocations, amplifications and methylations, cyclin D1, epidermal growth factor receptor (EGFR ), microsatellite instability and human papilloma virus (HPV) presence allelic loss on chromosomes 9p has been observed in oral cancer (Nawroz and Hruban, 2009).

Mitochondrial DNA mutations have also been useful targets to detect exfoliated oral squamous cell carcinoma (OSCC) cells in saliva, they have been identified in 46% of head and neck cancers, the same mitochondrial DNA mutations were detected in 67% of saliva samples from oral squamous cell carcinoma patients by direct sequencing alone (Fliss and caballero, 2008).

Level 2 Altered mRNA transcripts: RNA for years was thought to quickly degrade in saliva due to the various RNAses that saliva contains, de-spite the opposite reports, cell-free RNA molecules however, seem to existing saliva both intact but also fragmented (Zhou et al, 2008).

An intriguing question that remains to be answered is the mechanism by which mRNA in saliva is protected by degradation . A speculation is that salivary mRNA is contained in apoptotic bodies (Ratajczak and Wysoczynski, 2006). Various mRNA molecules were found up-regulated in the saliva of patients suffering from oral squamous cell carcinoma, seven mRNA molecule transcripts of (Li Y et al, 2004):

- IL8 (interleukin 8) playing a role in angiogenesis, replication, calcium-mediated signaling pathway, cell adhesion, chemo taxis, cell cycle arrest and immune response.
- IL1 (interleukin 1) which takes part in signal transduction; proliferation; inflammation and apoptosis.
- DUSP1 (dual specificity phosphatase 1) with a role in protein modification; signal transduction and oxidative stress.
- H3F3A (H3 histone, family 3A) having a DNA binding activity.
- OAZ1 (ornithine decarboxylase antizyme 1) taking part in polyamine bio synthesis.
- S100P (S100 calcium binding protein P) with a role in protein binding and calcium ion binding.
- SAT (spermidine/spermine N1-acetyltransferase) which takes part in enzyme and transferase activity- were found significantly elevated in oral squamous cell carcinoma patients rather than in healthy controls (Zimmermann and Wong, 2008).

**Level 3 Altered Protein:** Several salivary protein markers for oral squamous cell carcinoma have been investigated in various studies and have shown relatively moderate sensitivity and specificity values relative to prognosis prediction (Li Y et al, 2004), for example, defensins are peptides which possess antimicrobial and cytotoxic properties. They are found in the azurophil granules of polymorph nuclear leukocytes (Lehrer and Ganz, 2009). Elevated levels of salivary defensin-1 were found to be indicative for the presence of oral squamous cell carcinoma, since higher concentrations of salivary defensin-1 were detected in patients with oral squamous cell carcinoma compared with healthy controls (Zimmermann and Wong, 2008).

**1.13 Anti-oxidant of whole saliva**

In addition to various defense mechanism of saliva, the antioxidant system considered another important defense system found in saliva (Brock et al, 2004; Gorelik et al, 2007). Antioxidant whether they are enzymatic or non-enzymatic are found in high concentration in parotid saliva as compared to submandibular or sublingual saliva (Nagler et al, 2002). The most important antioxidants found in saliva are total protein, albumin, vitamin E, vitamin C, uric acid and malondialdehyde (MDA) and their concentrations in saliva of normal individual has been shown by various studies as shown in Table (1.14).

Salivary antioxidant system may influence the occurrence of dental caries through its effect on dental plaque metabolism, previously, it was found that antioxidant inhibited experimentally induced hamster caries which might be attributed to the inhibition of aerobic oxidative level (Burk and Wood, 1963; Lisanti and Eichel, 1963). Later on it has been shown that biofilm character of dental plaque allows for population diversity and coexistence of aerobic microorganism in addition to anaerobic and microaerophilic (Marquis, 1995), also it was found that different salivary antioxidants provide protection against Reactive Oxygen Species (ORS)-induced damage of periodontal tissues (Enwonwu, 1995; Koni and Navia, 1995). Dawes in 1996 found that the concentration of total protein in saliva was only about one-thirteen of that in plasma. Despite of this low concentration in saliva, but there are more than 40 proteins have been identified in saliva (Ritchel and Thompson, 1983; Mandel, 1989). About 65% of total salivary protein are proline-rich protein and about 25% of total protein concentration being α-amylase, lactoferрин, histatins, mucins, tissue growth factors, etc. all these play an important role in maintaining the oral health (Rudney, 1995; Hay and Bowen 1996).

Proteins are the major antioxidant in saliva, Meucci in 1998 showed that there is a good correlation between total protein concentration and salivary total antioxidant capacity. Several studies were done to investigate the relationship between salivary protein (antioxidant) and dental caries experience in that some studies shown that amino acids (lysine, methionine, and glutamine) did not inhibit experimental caries in cotton rats (Thompson et al, 1965), then some studies shown that a similar finding in that they found no association between salivary protein (cystatins) concentration and dental caries (Shomers et al, 1982), whereas another studies done by Tabak and his co-workers (1994) shown an inverse relationship between salivary protein (cystatins) concentration in resting whole saliva and active caries experience.

Concerning salivary proteins, albumin is one of salivary proteins with antioxidant capacities which is water soluble antioxidant and it is also found in gingival crevicular fluid, it provides minor contribution to salivary antioxidant system and its concentration in saliva is lower than that in serum (Moore et al, 1994; Sculey and Langley-Evan, 2002). In elderly subjects a relationship between root caries and serum albumin is highly possible in that there is
an inverse relationship between root caries and serum albumin level, so that persons with hypoalbuminemia are at high risk for root caries (Yoshihara et al, 2003).

In addition to the studies that were done to show the correlation between dental caries and salivary protein another studies were done to investigate the relationship between total protein and periodontal disease and these studies revealed different findings, in that several studies showed that both enzymatic (trypsin-like protease, elastase-like protease, general protease, glucosidase, and B-galactosidase) and non-enzymatic (platelet-activating factor, albumin, and cystatins) proteins, in addition to total protein were significantly higher in whole saliva of subjects with advanced periodontitis than those with healthy periodontium (Nieminen et al, 1993; Rasch et al, 1995; Henskens et al, 1996; Zuabi et al, 1999; Moynihan, 2000), another study was shown that the concentration of salivary albumin was similar in those with mild, no disease, moderate and severe periodontal disease as determined by community periodontal index and treatment need (CPITN), on the other hand another studies found that in elderly subjects (aged 75 years old) an inverse relationship between periodontal disease and serum albumin concentration were reported (Ogawa et al, 2006), similarly it has been shown than that serum albumin level is a significant risk predictor of periodontal disease progression among elderly persons (70 years old) (Iwasaki et al, 2008).

Vitamin C is another salivary antioxidant that is present in salivary aciner cells in a relatively higher concentration and involved in many cellular functions including pyrimidines metabolism, intracellular calcium, the catecholamines and other neurotransmitters which regulate salivary gland exocytosis (Enwonwu, 1992). The concentration of vitamin C in saliva is lower than its concentration in serum (Moore et al, 1994). However, its concentration in gingival crevicular fluid is three times higher than its concentration in plasma as it prevents activation of neutrophile derived collagenase in gingival crevicular fluid (Chapple, 1996). In comparison to uric acid, vitamin C provide minor contribution to salivary antioxidant system (Sculley and Langley-Evan, 2002; Panjamurthy et al, 2005; Sarai et al, 2005).

Several studies were done to investigate the correlation between ascorbic acid metabolism and dental caries experience and these studies were shown a controversy in that one of these studies found that the dental caries activity was higher among subjects who took 200 mg of vitamin C as a daily supplement for two years period as compared to control group (Grandison et al, 1942), another study shown that both blood and urine ascorbic acid levels were not significantly related to dental caries activity (DMFS index) in 341 systemically healthy males (Shannon and Gibson, 1964). Whereas the hypothesis that vitamin C has cariostatic effect was true to some extent.

Väänänen et al (1994) assumed that since ascorbic acid affects in vitro growth of bacteria and so may also act in vivo to decrease dental caries activity. They found that the amount of visible plaque and numbers of decayed tooth surfaces were significantly higher in the low ascorbic acid group than in the controls. Regarding vitamin E, it could affect dental caries occurrence through its immune enhancing effect (Meydani et al, 1990), in addition to its interaction with vitamin C (Chan, 1993).

During the first half of the previous century, studies carried on guinea pigs showed that vitamin C deficiency aggravate the existing gingival inflammation and periodontal pockets in addition to excessive bone resorption (Glickman, 1948; Hunat and Paynter, 1959). Later on Alfano et al (1975) and Nishida et al (2000) reported that severe vitamin C deficiency lead to a severe periodontal syndrome called "scorbutic gingivitis" which is characterized by ulcerative gingivitis, gingival bleeding and rapid periodontal pocket development with tooth exfoliation. Suggesting that vitamin C is essential for maintaining periodontal health. Similarly, the beneficial role of vitamin C in promoting periodontal health was reported by Rowe et al (1999).

They showed that treatment with calcium ascorbate containing vitamin C metabolites enhanced the formation of mineralized nodules and collagenous proteins. Also it was found that ascorbic acid promotes periodontal cells differentiation into osteoblasts and that ascorbic acid induced increased type collagen production and alkaline phosphatase activity (which is a specific marker associated with osteoblastic phenotypes) in periodontal cells (Ishikawa et al, 2004).

Evidence from epidemiological studies was controversial. Several studies reported no association between signs of past or present periodontal disease and the levels of serum ascorbic acid (Russell et al, 1961; Russell, 1963; Leggott et al, 1980; Chapple,
Uric acid is another non-proteinous antioxidant which is important an aqueous non-enzymatic antioxidant in human saliva (Moore et al, 1994). Its concentration in saliva is similar to that in serum and there is a great individual variation in salivary uric acid content (Kondakova et al, 1999). The antioxidant contribution of uric acid to gingival crevicular fluid has not been investigated (Chapple, 1996). A study done by Sculley and Langley-Evans (2003) shown that patient with sever periodontal disease show lower level of salivary uric acid antioxidant with increased oxidative damage within the oral cavity as compared to those with mild or no periodontal disease as determined by CPITN index.

Malondialdehyde (MDA) is an important product of lipid peroxidation that will occur as a result of oxidative stress and impaired salivary antioxidant capacity (Battino et al, 2002; Rai et al, 2006). The level of salivary malondialdehyde (MDA) will be elevated in patient with dental caries as compared with those who are caries free (control group) though the difference was not statistically significant. This finding is partially confirmed by Suntsov et al (2008) study, they showed that dental caries progression is accomplished by activation of free radical oxidation.

The effect of lipid peroxidation in periodontal disease pathogenesis can be established by determining the level of MDA in saliva, in that many studies showed that the level of salivary MDA was elevated in patient with periodontitis which indicate the involvement of lipid peroxidation in periodontal disease pathogenesis (Gambhir et al, 1997; DeZewart et al, 1999). Also data suggests that Thiobarbituric Acid Reactive substances (TBARs) (which reflects MDA production) level in gingival tissues is closely associated with periodontal status (Tuter et al, 2001). Similarly, another study reported that patients with periodontitis had a significantly higher salivary TBARs level than healthy control (Panjamurthy et al, 2005).
Table (1.14): Review of studies concerning the concentration of antioxidants in whole saliva of normal individuals.

<table>
<thead>
<tr>
<th>Author</th>
<th>Origin</th>
<th>Age</th>
<th>Type of saliva</th>
<th>Mean±SD</th>
</tr>
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<tr>
<td><strong>Total protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Jaafery, 1999</td>
<td>Iraq</td>
<td>30-65</td>
<td>Unstimulated</td>
<td>141.82±39.18</td>
</tr>
<tr>
<td>Abass, 2006</td>
<td>Iraq</td>
<td>35-45</td>
<td>Unstimulated</td>
<td>121.8±52.6</td>
</tr>
<tr>
<td>Daoud, 2008</td>
<td>Iraq</td>
<td>15-75</td>
<td>Unstimulated</td>
<td>221.0±62.0</td>
</tr>
<tr>
<td>Al-Saadi, 2009</td>
<td>Iraq</td>
<td>6-11</td>
<td>Stimulated</td>
<td>48.0±20.0</td>
</tr>
<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>2044.40±111.10</td>
</tr>
<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>1044.40±88.88</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
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<tr>
<td>Daoud, 2008</td>
<td>Iraq</td>
<td>15-75</td>
<td>Unstimulated</td>
<td>27.0±19.0</td>
</tr>
<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>22.60±12.22</td>
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<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>20.63±13.11</td>
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<tr>
<td><strong>Vitamin E</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sarai et al, 2005</td>
<td>Turkey</td>
<td>23-39</td>
<td>Stimulated</td>
<td>0.04±0.011</td>
</tr>
<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>0.89±0.00</td>
</tr>
<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
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<tr>
<td>Sarai et al, 2005</td>
<td>Turkey</td>
<td>23-39</td>
<td>Stimulated</td>
<td>0.104±0.011</td>
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<td>Yas, 2009</td>
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<td>Unstimulated</td>
<td>7.59±0.00</td>
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<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>0.73±0.00</td>
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<tr>
<td><strong>Uric acid</strong></td>
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<tr>
<td>Atiyah, 2008</td>
<td>Iraq</td>
<td>45-75</td>
<td>Unstimulated</td>
<td>3.53±0.48</td>
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<td>Daoud, 2008</td>
<td>Iraq</td>
<td>15-75</td>
<td>Unstimulated</td>
<td>2.31±1.31</td>
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<td>Yas, 2009</td>
<td>Iraq</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>8.89±3.36</td>
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<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>8.75±3.71</td>
</tr>
<tr>
<td><strong>Malondialdehyde (DMA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rai et al, 2006</td>
<td>India</td>
<td>15-60</td>
<td>Unstimulated</td>
<td>Male=0.05±0.004 Female=0.04±0.007</td>
</tr>
<tr>
<td>Atiyah, 2008</td>
<td>Iraq</td>
<td>45-75</td>
<td>Unstimulated</td>
<td>0.23±0.07</td>
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<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>30-40</td>
<td>Unstimulated</td>
<td>0.87±0.00</td>
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<tr>
<td>Yas, 2009</td>
<td>Iraq</td>
<td>55-65</td>
<td>Unstimulated</td>
<td>0.67±0.00</td>
</tr>
</tbody>
</table>
Conclusion

Saliva is one of many body fluids, secreted in the oral cavity and play an important role in maintaining the integrity of oral hard and soft tissue, saliva can perform a number of protective functions to the oral cavity as well as to the body through its complex physical and chemical composition.

- Salivary flow rate differ between stimulated and unstimulated saliva in that it is faster in stimulated saliva than in unstimulated saliva.
- Changes in the salivary flow rate and composition of saliva may result in a significant oral health problems such as xerostomia.
- One of the most important functions of saliva is protection of tooth against dissolution which is caused by cariogenic challenge or by dental erosion, this is done by controlling the pH of the oral environment by means of secreted bicarbonate ion and maintaining super saturation state with respect to mineral phase.
- There is a controversy in investigating the relationship between the concentrations of inorganic constituents of saliva and dental caries in that some show a positive correlation, some show a negative correlation while the others show that there is no relation.
- Saliva can be used as a biomarker of oral cancer.
- Saliva containing an antioxidant which is considered an additional defense mechanism and play an important role in influencing the occurrence of dental caries in human whole saliva.

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