1 Introduction and Literature Review

1.1 Tobacco

1.1.1 Tobacco definition

Tobacco is a product processed from the dried leaves of plants in the genus Nicotiana. It can be used as a pesticide, and extracts form ingredients of some medicine (Reddy *et al*., 1980), but is most commonly consumed as a drug. Tobacco is a name for any plant of the genus Nicotiana of the Solanaceae family (nightshade family) and for the product manufactured from the leaf used in cigars and cigarettes, snuff, pipe tobacco, chewing tobacco, and flavored shisha. The health effects of tobacco are the circumstances mechanisms, and factors of tobacco consumption on human health, epidemiological research has been focused primarily on cigarette tobacco smoking, smokeless tobacco (snuff) and both(smoking and snuff) which has been studied more extensively than any other form of consumption (Robert, 2010).

1.1.2 Tobacco Classification

1.1.2.1 Tobacco smoking

Commercially manufactured cigarettes are seemingly simple objects consisting of mainly tobacco blend which may contain over 100 ingredients including tar and fla-vourants. Tar, a term used to describe the toxic chemicals found in cigarette is present in all cigarettes and tends to increase as the cigarette is burnt down. Tar in cigarette paralyzes the cilia in the lungs and contribute to lung diseases such as emphysema, bronchitis, lung cancer (Tigni, 1993).

1.1.2.2 Smokeless Tobacco

The two main types of smokeless tobacco in the United States are chewing tobacco and snuff. Chewing tobacco comes in the form of loose leaf, plug, or twist. Snuff is finely ground tobacco that can be dry, moist, or in sachets (tea bag-like pouches) (Nagler *et al*., 2000).

1.1.3 Route of administration

Smoking tobacco comes in form of cigarette or hookahs or pipe, Snuffing Snuff is finely ground tobacco that can be dry, moist, or in sachets (tea bag-like pouches),and Chewing tobacco comes in the form of loose leaf, plug , or twist. Snuff is finely ground tobacco that can be dry, moist, or in sachets (tea bag-like pouches). Although some forms of snuff can be used by sniffing or inhaling into the nose, most smokeless tobacco users place the product in their cheek or between their gum and cheek. Users then suck on the tobacco and spit out the tobacco juices, which is why smokeless tobacco is often

referred to as spit or spitting tobacco. The nicotine in this tobacco is absorbed (Nagler *et al.*, 2000).

1.1.4 Complications of abuse

Cardiovascular pathologies (heart attack), pleuropneumonia, Cancers, Stroke, leukoplakia (a lesion of the soft tissue in the mouth that consists of a white patch or plaque that cannot be scraped off) and recession of the gums, chronic obstructive pulmonary disease and hypertension (Martins, 2008).

1.2 Calcium biochemistry and physiology

Calcium is the fifth most common element in the human body. It is an essential element that is only available to the body through dietary sources (Munro, 2010). The main sources of calcium are: mill, cheese, eggs, fish, green vegetables and fruits (Kamal, 2007). Current dietary calcium recommendations range from1000 to 1500mg/d, depending on age. In some individuals, particularly the elderly, calcium supplements may be needed to achieve the recommended dietary calcium intake. Calcium plays a key role in a wide range of biologic functions, either in the form of its free ion or bound complexes. One of the most important functions as bound calcium is in skeletal mineralization. The vast majority of total body calcium (99%) is present in the skeleton as calcium-phosphate complexes, primarily as hydroxyl apatite, which is responsible for much of the material properties of bone. In bone, calcium serves two main purposes: it provides skeletal strength and, concurrently, provides a dynamic store to maintain the intra- and extracellular calcium pools. Non bone calcium represents 1% of total body calcium. However, it is constant and rapid exchange within the various calcium pools and is responsible for a wide range of essential functions, including extra- and intracellular signaling, nerve impulse transmission, and muscle contraction (Munro, 2010).

Calcium also plays a major signaling role in the body. This is true for closely regulated extracellular calcium, a decrease of which will trigger, among others, release of parathyroid hormone, a molecule that by its action on bone tends to restore plasma calcium to 2.5 mmol/L, the concentration aimed for in calcium homeostasis, calcium also plays an important role as intracellular messenger in many systems and cells (e.g., cardiac, renal) (Felix and Danielle, 1988).

Calcium is necessary to stabilize or allow maximal activity for a number of blood clotting enzymes. However, the normal activity of these enzymes is not significantly affected by changes in extracellular calcium concentrations or by dietary calcium deficiency, and is practically independent of dietary calcium intake. Also there is good consensus on the role of

calcium in the stabilization and activity of certain enzymes involved in energy metabolism, such as glyceraldehydes phosphate dehydrogenase, pyruvate dehydrogenase, and ketoglutarate dehydrogenase (European Food Safety Authority, 2009).

Dietary calcium plays a pivotal role in the regulation of energy metabolism because high-calcium diets attenuate adipocyte lipid accretion and weight gain during the overconsumption of an energy dense diet and increase lipolysis and preserve thermogenesis during caloric restriction, which thereby calcium markedly accelerates weight loss. Intracellular calcium plays a key regulatory role in adipocyte lipid metabolism and triacylglycerol storage; increased intracellular calcium results in the stimulation of lipogenic gene expression and lipogenesis and the suppression of lipolysis, which results in increased lipid filling and increased adiposity (Michael, 2004).

Calcium also plays role in insulin secretion and constriction and relaxation of blood vessels (Kamal, 2007).

1.2.1 Calcium Distribution

About 99% of Ca^{2+} in the body is part of bone. The remaining1% is mostly in the blood and other ECF. Little is in the cytosol of most cells. In fact, the concentration of ionized Ca^{2+} in blood is 5,000 to 10,000 times higher than in the cytosol of cardiac or smooth muscle cells. Maintenance of this large gradient is vital to maintain the essential rapid inward flux of Ca^{2+}. Ca^{2+} in blood is distributed among several forms.

About 45% circulates as free Ca^{2+} ions (referred to as ionized Ca^{2+}), 40% is bound to protein, mostly albumin, and 15% is bound to anions, such as $HCO3^-$, citrate, $PO4^-$, and lactate. Clearly, this distribution can change in disease. It is noteworthy that concentrations of citrate, $HCO3^-$, lactate, $PO4^-$, and albumin can change dramatically during surgery or critical care. This is why ionized Ca^{2+} cannot be reliably calculated from total Ca^{2+} measurements, especially in acutely ill individuals (Michael et al., 2010).

1.2.2 Calcium regulation

There are many factors that regulate serum calcium level:

1.2.2.1 Parathyroid Hormone (PTH)

The biological action of PTH includes:

A. Stimulation of osteoclastic bone resorption and release of calcium and phosphate from bone.

B. Stimulation of calcium reabsorption and inhibition of phosphate reabsorption from the renal tubules.

C. Stimulation of renal production of 1,25(OH)2D3, which increases intestinal absorption of calcium and phosphate.(Gregory and Theresa.,1999)

1.2.2.2 Vitamin D

Vitamin D increases plasma calcium and phosphate concentrations by increasing the absorption of calcium and phosphate from gastrointestinal tract. It also increases bone resorption and enhances the effects of PTH in the nephron to promote renal tubular calcium reabsorption (Gregory and Theresa, 1999). Vitamin D and calcium were highly correlated with a strong positive correlation (Katherine *et al*., 2009).

1.2.2.3 Calcitonin

Calcitonin is a 32-amino acid peptide that is synthesized and secreted by the parafollicular cells of the thyroid gland (Gregory and Theresa, 1999). It is secreted when the concentration of Calcium in the blood increases. Calcitonin exerts its calcium-lowering effect by inhibiting the actions of both PTH and vitamin D. Although calcitonin is apparently not secreted during normal regulation of the ionized calcium concentration in blood, it is secreted in response to a hypercalcemic stimulus (Michael *et al*., 2004).

1.2.2.4 Thyroid hormone

It secreted from thyroid gland in form of T3 and T4.It acts to increase releasing of calcium and phosphate from bone.

1.2.2.5 Alkaline Phosphatase enzymes (ALP)

ALP secrete from bony osteoblast. It reduces calcium level by bone calcification (Kamal A., 2007).

1.2.3 Calcium pathology

Low total serum calcium (hypocalcemia) may be due to either a reduction in the albumin-bound calcium, the free fraction of calcium, or both. Hypoalbuminemia is the most common cause of decreased total calcium with normal free calcium (sometimes called pseudohypocalcemia); serum calcium is lower when serum albumin is low because 1 g/dL of albumin binds approximately 0.8 mg/dL of calcium. Common clinical conditions associated with low serum albumin include chronic liver disease, nephrotic syndrome, congestive heart failure, and malnutrition (Carl *et al.,* 2008).

In chronic renal failure, hypoproteinemia, hyperphosphatemia, low serum $1,25(OH)_2D$ (reduced synthesis because of inadequate renal mass), and skeletal resistance to PTH contribute to hypocalcemia. Magnesium deficiency, as discussed in a later section of this chapter, impairs PTH secretion and causes PTH end-organ resistance (Carl *et al.,* 2008).

Hypoparathyroidism is due most commonly to parathyroid gland destruction during neck surgery (90%). Pseudohypoparathyroidism is characterized by resistance to PTH and increased concentrations of PTH (Carl *et al.,* 2008).

Rapid remineralization of bone after surgery for primary hyperparathyroidism (hungry bone syndrome), treatment for hyperthyroidism, or treatment for hematological malignancy may result in hypocalcemia. Acute pancreatitis is frequently complicated by hypocalcemia. Vitamin D deficiency may also be associated with hypocalcemia because of impaired intestinal absorption of calcium and skeletal resistance to PTH (Carl *et al.,* 2008). Hypocalcemia most commonly presents with signs and symptoms of neuromuscular hyper excitability, such as tetany, paresthesia, and seizures. A rapid fall in the serum calcium also may be associated with hypotension and ECG abnormalities (Carl *et al.,* 2008).

The initial laboratory evaluation includes assessment of renal function and measurement of serum albumin and magnesium concentrations. Serum intact PTH concentrations are low or inappropriately normal in hypoparathyroidism and elevated

in pseudohypoparathyroidism. Vitamin D deficiency is characterized by low serum 25(OH)D, high PTH (secondary hyperparathyroidism), and high serum alkaline phosphatase (ALP). For symptomatic hypocalcemia, calcium may be administered intravenously (Carl *et al.,* 2008).

1.2.3.1 Causes of Hypocalcemia

Primary hypoparathyroidism glandular aplasia destruction, or removal, hypomagnesemia, hypermagnesemia, hypoalbuminemia (total calcium only, ionized not affected by)—chronic liver disease, nephritic syndrome, malnutrition, acute pancreatitis vitamin D deficiency, Renal disease, Rhabdomyolysis, and Pseudohypoparathyroidism (Michael *et al*., 2010).

1.2.3.2 Hypercalcemia

Hypercalcemia is commonly encountered in clinical practice and results when the flux of calcium into the extracellular fluid compartment from the skeleton, intestine, or kidney is greater than the efflux. Hypercalcemia is caused by (1) increased intestinal absorption, (2) increased renal retention, (3) increased skeletal resorption, or (4) a combination of mechanisms (Carl *et al.,* 2008).

Primary hyperparathyroidism is the most common cause in outpatients, whereas malignancy is the most common cause in hospitalized patients. Together, these two disorders account for 90% to 95% of all cases of hypercalcemia (Carl *et al.,* 2008).

Primary hyperparathyroidism is most often caused by an adenoma, but may be caused by hyperplasia involving multiple parathyroid glands, or, rarely, by parathyroid carcinoma (Carl *et al*., 2008).

Greater than 80% of hyperparathyroid patients are relatively free of symptoms on presentation because of the early detection of this disorder by the widespread use of chemistry testing panels that include calcium. The most common signs and symptoms of hypercalcemia are nonspecific and related to the neuromuscular system. They include fatigue, malaise, and weakness with mild hypercalcemia (calcium < 12 mg/dl); depression, apathy, and inability to concentrate may be present at higher concentrations. Hypercalcemia may induce mild nephrogenic diabetes insipidus with increased thirst and increased urination. Chronic hypercalcemia with hypercalciuria has been known to lead to formation of calcium-containing kidney stones, which, in some cases, leads to slowly developing renal failure. The majority of patients with primary hyperparathyroidism (>60%) are postmenopausal women (Carl *et al.*, 2008).

Hypercalcemia can be primary or secondary; primary due to Primary hyperparathyroidism is diagnosed by laboratory studies. Hypercalcemia should be documented by measuring total calcium and serum albumin, or ideally free calcium, on more than one occasion before initiating further testing. Measurement of intact PTH (with concomitant measurement of calcium) is the most sensitive and specific test for parathyroid function and is central to the differential diagnosis of hypercalcemia. In parathyroid-related hypercalcemia, plasma PTH is not suppressed (below its reference interval); in other causes of hypercalcemia, the high calcium suppresses PTH production by the parathyroid glands. Serum $1,25(OH)_2D$ is usually above the middle of the reference interval in primary hyperparathyroidism, as PTIH stimulates its production. By contrast, $1,25(OH)_2D$ (like PTH) is low-normal or suppressed in nonparathyroid hypercalcemia, except in sarcoidosis, other granulomatous diseases, and certain lymphomas in which the pathological tissues contain the 25-hydroxyvitamin D-1α-hydroxylase required to produce $1,25(OH)_2D$ (Carl *et al.*,2008).

1.2.3.3 Causes of Hypercalcemia

Primary hyperparathyroidism adenoma or glandular, hyperplasia, Hyperthyroidism, Benign familial hypocalciuria, Malignancy, Multiple myeloma, Increased vitamin D, Thiazide diuretics and Prolonged immobilization (Michael *et al*., 2010).

1.3 Phosphate biochemistry and physiology

Phosphorus in the form of inorganic and organic phosphate is an important and widely distributed element in the human body. Inorganic phosphate is the fraction measured in serum and pleura by clinical laboratories (Carl *et al.*, 2008).

Phosphate is found everywhere in living cells, phosphate compounds participate in many of the most important biochemical processes. The genetic materials deoxyribonucleic acid

(DNA) and ribonucleic acid (RNA) are complex phosphodiesters. Most coenzymes are esters of phosphoric or pyrophosphoric acid. The most important reservoirs of biochemical energy are ATP, creatine phosphate, and phosphoenolpyruvate. Phosphate deficiency can lead to ATP depletion, which is ultimately responsible for many of the clinical symptoms observed in hypophosphatemia. Alterations in the concentration of 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells affect the affinity of hemoglobin for oxygen, with an increase facilitating the release of oxygen in tissue and a decrease making oxygen bound to hemoglobin less available. By affecting the formation of 2,3-BPG, the concentration of inorganic phosphate indirectly affects the release of oxygen from hemoglobin. Understanding the cause of an altered phosphate concentration in the blood is often difficult because transcellular shifts of phosphate are a major cause of hypophosphatemia in blood. That is, an increased shift of phosphate into cells can deplete phosphate in the blood. Once phosphate is taken up by the cell, it remains there to be used in the synthesis of phosphorylated compounds. As these phosphate compounds are metabolized, inorganic phosphate slowly leaks out of the cell into the blood, where it is regulated principally by the kidney (Michael *et al.*, 2010).

Although the concentration of all phosphate compounds in blood is about 12 mg/dL (3.9 mmol/L), most of that is organic phosphate and only about 3 to 4 mg/dL is in organic phosphate. Phosphate is the predominant intracellular anion, with intracellular concentrations varying, depending on the type of cell. About 80% of the total body pool of phosphate is contained in bone, 20% in soft tissues, and less than 1% is active in the serum/plasma (Michael *et al*., 2010).

1.3.1 Phosphate regulation

Phosphate in blood may be absorbed in the intestine from dietary sources, released from cells into blood, and lost from bone. In healthy individuals, all these processes are relatively constant and easily regulated by renal excretion or reabsorption of phosphate. Disturbances to any of these processes can alter phosphate concentrations in the blood; however, the loss of regulation by the kidneys will have the most profound effect. Although other factors, such as vitamin D, calcitonin, growth hormone, and acid-base status, can affect renal regulation of phosphate, the most important factor is PTH, which overall lowers blood concentrations by increasing renal excretion (Michael *et al*., 2010).

Vitamin D acts to increase phosphate in the blood, thus vitamin D increases both phosphate absorption in the intestine and phosphate reabsorption in the kidney. Growth hormone, which helps regulate skeletal growth, can affect circulating concentrations of phosphate. In cases of

excessive secretion or administration of growth hormone, phosphate concentrations in the blood may increase because of decreased renal excretion of phosphate (Michael *et al.*, 2010).

1.3.2 Phosphate pathology

Hypophosphatemia, defined as the concentration of inorganic phosphate in the serum below the normal reference interval, usually < 2.5 mg/dL (< 0.81 m mol/L), is relatively common in hospitalized patients (approximately 2%). Hypophosphatemia may be present when cellular concentrations are normal, and cellular phosphate depletion may exist when serum concentrations are normal or even high. Hypophosphatemia or phosphate depletion may be caused by (1) a shift of phosphate from extracellular to intracellular spaces, (2) renal phosphate wasting, (3) decreased intestinal absorption, and (4) loss from intracellular phosphate (Carl *et al.,* 2008).

Injected insulin and carbohydrate-induced stimulation of insulin secretion increase the transport of phosphate and glucose into cells and thus are common causes of hypophosphatemia. Re-feeding of malnourished individuals causes an intracellular shift of phosphate. Respiratory alkalosis leads to an increase in intracellular pH, which activates phosphofructokinase and accelerates glycolysis, causing a shift of phosphate into the cell (Carl *et al.,* 2008).

In some instances, (1) excessive PTH secretion, (2) Fanconi syndrome, (3) X-linked hypophosphatemic rickets, and (4) tumor-induced osteomalacia will result in loss of phosphate in urine and may also cause hypophosphatemia or phosphate depletion. Hypophosphatemia or phosphate depletion due to inadequate phosphate absorption is less common given the abundance of phosphate in the diet, hut may occur in individuals taking aluminum- or magnesium-containing antacids and in patients with malabsorption. The antacids bind phosphate, thus hindering its absorption. The hypophosphatemia and phosphate depletion in patients with malabsorption may be more closely related to their secondary hyperparathyroidism (and resulting loss of phosphorus in urine) than to malabsorption of phosphate (Carl *et al.*, 2008).

Intracellular phosphate may be lost in acidosis as a result of the catabolism of organic compounds within the cell. Diabetic ketoacidosis is associated initially with high-normal to increased serum phosphate. Treatment of the ketosis and acidosis with insulin and intravenous fluids, however, results in a rapid decrease in the serum phosphate concentration. Consequently, patients being treated for diabetic ketoacidosis may have both intracellular phosphate depletion and hyperphosphatemia. The clinical manifestations of serum phosphate depletion depend on the length and degree of the deficiency. Plasma concentrations < l.5

mg/dL (< 0.48 mmol/L) may produce clinical manifestations. Because phosphate is necessary for the formation of ATP, both glycolysis and cellular function are impaired by low intracellular phosphate concentrations. Muscle weakness, acute respiratory failure, and decreased cardiac output may occur in phosphate depletion. At very low serum phosphate (< l mg/dL or < 0.32 mmol/L), rhabdomyolysis may occur. Phosphate depletion in erythrocytes decreases erythrocyte 2,3-diphosphoglycerate, which causes tissue hypoxia because of increased affinity of hemoglobin for oxygen. Severe hypophosphatemia (serum phosphate concentration < 0.5 mg/dL [< 0.l6 mmol/L]) may result in hemolysis of red blood cells. Mental confusion and frank coma also may be secondary to the low ATP and tissue hypoxia. If hypophosphatemia is chronic, rickets (in children) and osteomalacia (in adults) may develop (Carl *et al.,* 2008).

Treatment of hypophosphatemia depends on the degree of hypophosphatemia and the presence of symptoms. Patients with moderate hypophosphatemia may require only treatment of the underlying disorder or oral phosphate supplementation. In patients with severe symptoms of hypophosphatemia, particularly if respiratory muscle weakness is present, parenteral administration of phosphate may be indicated (Carl *et al.,* 2008).

1.3.3 Causes of Hypophosphatemia and Phosphate Depletion

Primary or secondary hyperthyroidism, Renal tubular defect (Familial hypophosphatemia and Fanconi syndrome), decreased intestinal phosphate absorption, Increase loss (Diarrhea) and Decreased absorption (malabsorption and Vitamin D deficiency). Hyperphosphatemia is usually secondary to the inability of the kidneys to excrete phosphate, as in renal failure. Moderate increases of serum phosphate occur in individuals with (1) low PTH (hypoparathyroidism), (2) PTH resistance (pseudohypoparathyroidism), or (3) acromegaly (increased growth hormone) caused by an increased renal phosphate threshold. A rapid increase in serum phosphate may be associated with hypocalcemia. Therefore symptoms may include tetany, seizures, and hypotension. Long-term hyperphosphatemia may be associated with first secondary hyperparathyroidism, second osteitis fibrosa, and third soft tissue calcification of the kidneys, blood vessels, cornea, skin, and periarticular tissue (Carl *et al.,* 2008).

Therapy for hyperphosphatemia is directed toward correcting the cause of high serum phosphate. In renal failure and in hypoparathyroidism, dietary restriction of phosphate and agents that bind phosphate in the intestine (calcium carbonate and others) are useful in lowering the serum phosphate concentrations (Carl *et al.,* 2008).

1.3.4 Causes of Hyperphosphatemia

Decreased renal phosphate excretion, Decreased glomerular filtration rate, Chronic and acute renal failure, Increased tubular reabsorption, hypoparathyroidism, Pseudo hypoparathyroidism, Acromegoly, Increase phosphate intake oral or intravenous administration, Lactic acidosis and Respiratory acidosis (Carl *et al.*, 2008).

1.4 Magnesium

Magnesium is the fourth most abundant cation in the body. Approximately 55% of the total body magnesium is in the divalent skeleton and 45% is intracellular where it is the most prevalent cation (Carl *et al.*, 2008).

1.4.1 Biochemistry and Physiology

Magnesium (Mg^{+2}) is the fourth most abundant cation in the body and second most abundant intracellular ion. The average human body (70 kg) contains 1 mole (24 g) of Mg^{+2}. Approximately 53% of Mg^{+2} in the body is found in bone, 46% in muscle and other organs and soft tissue, and less than 1% is present in serum and red blood cells.15 Of the Mg^{+2} present in serum, about one third is bound to protein, primarily albumin. Of the remaining two thirds, 61% exists in the free or ionized state and about 5% is complexes with other ions, such as PO_4^- and citrate. Similar to Ca^{2+}, it is the free ion that is physiologically active in the body. The role of Mg^{+2} in the body is widespread. It is an essential cofactor of more than 300 enzymes, including those important in glycolysis, transcellular ion transport, neuromuscular transmission, synthesis of carbohydrates, proteins, lipids, and nucleic acids, and release of and response to certain hormones. The clinical usefulness of serum Mg^{+2} levels has greatly increased in the past 10 years as more information about the analyte has been discovered. The most significant findings are the relationship between abnormal serum Mg^{+2} levels and cardiovascular, metabolic, and neuromuscular disorders. Although serum levels may not reflect total body stores of Mg^{+2}, serum levels are useful in determining acute changes in the ion (Michael *et al.*, 2010).

1.4.2 Regulation

Rich sources of Mg^{+2} in the diet include raw nuts, dry cereal, and “hard” drinking water; other sources include vegetables, meats, fish, and fruit. Processed foods, a never-increasing part of the average U.S. diet, have low levels of Mg^{+2} that may cause an inadequate intake. This in turn may increase the likelihood of Mg^{+2} deficiencies. The small intestine may absorb 20%–65% of the dietary Mg^{+2}, depending on the need and intake (Michael *et al.*, 2010).

The overall regulation of body Mg^{+2} is controlled largely by the kidney, which can reabsorb Mg^{+2} in deficiency states or readily excrete excess Mg^{+2} in overload states. Of the non protein-

bound Mg^{+2} that gets filtered by the glomerulus, 25%–30% is reabsorbed by the proximal convoluted tubule (PCT), unlike Na^{+}, in which60%–75% is absorbed in the PCT. Henley's loop is the major renal regulatory site, where 50%–60% of filtered Mg^{+2} is reabsorbed in the ascending limb. In addition, 2%–5% is reabsorbed in the distal convoluted tubule. The renal threshold for Mg^{+2} is approximately 0.60–0.85 mmol/L (_1.46–2.07 mg/dL). Because this is close to normal serum concentration, slight excesses of Mg^{+2} in serums are rapidly excreted by the kidneys. Normally, only about 6% of filtered Mg^{+2} are excreted in the urine per day (Michael et *al*., 2010).

Mg^{+2} regulation appears to be related to that of Ca^{2+} and Na^{+} Parathyroid hormone (PTH) increases the renal reabsorption of Mg^{+2} and enhances the absorption of Mg^{+2} in the intestine. However, changes in ionized Ca^{2+} have a far greater effect on PTH secretion. Aldosterone and thyroxine apparently have the opposite effect of PTH in the kidney, increasing the renal excretion of Mg^{+2} (Michael *et al*., 2010).

1.4.3 Magnesium Pathology

Hypomagnesemia is common in hospitals. Ten percent of the patients admitted to city hospitals and as many as 65% of patients in intensive care units may be hypomagnesemic. In many cases, the hypomagnesemia appears to reflect a shift into cells because it resolves without magnesium replacement. Moderate or severe magnesium deficiency is usually due to losses of magnesium from the gastrointestinal (GI) tract or kidneys. Magnesium deficiency is commonly associated with losses from the lower intestine in diarrhea. Because magnesium is most efficiently absorbed from the distal small bowel, malabsorption and bypass surgery for obesity are also associated with magnesium malabsorption. Nasogastric suction or vomiting may deplete body stores of magnesium as upper GI fluids contain approximately 0.5 mmol/L of magnesium (Carl *et al.,* 2008).

Excessive urinary losses of magnesium from the kidneys are important causes of magnesium deficiency in alcoholism and diabetes mellitus (osmotic diuresis) and with loop diuretics (furosemide) and aminoglycoside antibiotics. Increased sodium excretion (parenteral fluid therapy) and increased calcium excretion (hypercalcemia) also result in renal magnesium wasting (Carl *et al.,* 2008).

Neuromuscular hyper excitability with tetany and seizures may be present. Magnesium deficiency impairs PTH secretion and causes end-organ resistance to PTH, which may result in hypocalcemia. Cardiac arrhythmias have been associated with magnesium deficiency and are partly caused by the hypokalemia and intracellular potassium depletion that occurs in magnesium deficiency (Carl *et al.,* 2008). Hypomagnesemia is not necessarily an indication of

magnesium deficiency. Conversely, intracellular magnesium depletion and magnesium deficiency may exist despite a normal serum magnesium concentration (*Carl et al.,* 2008). Acute symptomatic magnesium deficiency is usually treated with parenteral magnesium; mild depletion may be treated with oral magnesium (Carl *et al., 2008*).

1.4.4 Causes of Magnesium Deficiency

Reduced intake (Poor diet/starvation, Prolonged magnesium-deficient IV therapy and Chronic alcoholism), decreased absorption (Malabsorption syndrome, Surgical resection of small intestine, Nasogastric suction, Pancreatitis, Vomiting, Diarrhea, Laxative abuse, Neonatal and Primary Congenital), increased excretion—renal (Tubular disorder, Glomerulonephritis and Pyelonephritis), increased excretion—endocrine, (Hyperparathyroidism, Hyperaldosteronism, Hyperthyroidism, Hypercalcemia and Diabetic ketoacidosis), increased excretion—drug induced (Diuretics, Antibiotics, Cyclosporin and Digitalis) and miscellaneous (Excess lactation and Pregnancy) (Michael *et al.*, 2010).

1.4.5 Hypermagnesemia

Magnesium intoxication is not common, although serum magnesium concentrations may, it mildly or moderately increased in as many as 12% of hospital patients. Symptomatic hypermagnesemia is usually caused by excessive intake, resulting from the administration of antacids, enemas, and parenteral fluids containing magnesium. Most symptomatic patients have concomitant renal failure, which limits the ability of the kidneys to excrete excess magnesium. Magnesium is a standard therapy for pregnancy-induced hypertension (preeclampsia and eclampsia); magnesium intoxication may be seen in mothers and their neonates (Carl *et al.,* 2008).

Depression of the neuromuscular system is the most common manifestation of magnesium intoxication. Deep tendon reflexes disappear at a serum magnesium above 5 to 9 mg/dL (2.06 to 3.70 mmol/L), whereas depressed respiration and apnea, caused by voluntary muscle paralysis, may occur at serum magnesium concentrations >I0 to 12 mg/dL b4.11 to 4.94 mmol/L), with cardiac arrest at even higher concentrations. Because calcium acutely antagonizes the toxic effects of magnesium, patients with severe magnesium intoxication may be treated with intravenous calcium. If necessary, peritoneal dialysis or hemodialysis against a low-dialysis magnesium bath effectively lowers the serum magnesium concentration (Carl *et al.,* 2008).

1.4.6 Causes of Hypermagnesemia

Causes of hypermagnesmia are decreased excretion, acute or chronic renal failure, hypothyroidism, hypoaldosteronism, hypopituitarism (↓GH), increased intake, Antacids,

Enemas, Cathartics, Therapeutic eclampsia, cardiac arrhythmia, Miscellaneous, Dehydration, Bone carcinoma and Bone metastases (Michael *et al.*, 2010).

1.5 The saliva

The saliva is a glandular secretion; it is not one of the popular bodily fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears (Streckifus and Bigler, 2002). Saliva plays a critical role in the maintenance of oral and dental health. Whole saliva represents a mixture of oral fluids and includes secretions from both major and minor salivary glands (90% of saliva is produced by the major salivary gland) (Mader, 2001), approximately 10% produced by minor glands clustered in the oral mucosa) in addition to several constituents of non-salivary origin , such as gingival crevicular fluid (GCF), serum and blood derivatives from oral wounds, bacteria and bacteria secretions, viruses and fungi, desquamated epithelial cells, other cellular components and food debris (Sreeby, 1989). Saliva can be collected with or without stimulation (Aristids *et al.*, 2013). Stimulated saliva is collected by masticatory action (i.e., from a subject chewing on paraffin wax) or by gustatory stimulation (i.e., application of citric acid on the subject's tongue) (Aristids *et al.*, 2013).

1.5.1 Composition of Saliva

Composition of saliva depends on various factors such as, the flow rate, type of gland and diet. Saliva is 99.5% water and 0.5% solute (John, 1998). Among the solute are: Inorganic ions such as, Na+, K+, Cl-, HCO3-, Ca2+,Mg2+, HPO4-2, I-, SCN- and F- (John, 1998). Proteins such as enzymes, antibacterial substances, albumin, blood clotting factors, B2-microglobulin and immunoglobulins, amino acids, prolin-rich proteins. And also the saliva contain small organic molecules such as cyclic AMP, and cyclic AMP binding proteins, urea, uric acid various lipids and corticosteroids (John, 1998). There are several ways by which serum constituents that are not part of the normal salivary constituents can reach saliva. Within the salivary glands; transfer mechanisms include intracellular and extracellular routes. The most common intracellular route is passive diffusion, although active transport has also been reported. Ultra filtration, which occurs through the tight junctions between the cells, is the most common extracellular route (Haeckel and Hanecke, 1996). Serum constituents are also found in whole saliva as a result of GCF out flow. Depending on the degree of inflammation in the gingival, GCF is either a serum transudate or, more commonly, inflammatory exudates that contain serum constituents (Kaufman and Lamster, 2000).

1.5.2 Major Functions of Saliva

Lubrication and fluid coating Mucins, proline-rich proteins and H_2O components of saliva provide physical protection against mechanical, thermal, chemical irritation Aids in speech

and swallowing Cleaning. The H_2O component facilitates clearance of food and swallowing and bathing the teeth. Remineralization Super saturation of calcium and phosphate due to the presence of the salivary protein (statherin) facilitates remineralization of the teeth. Anionic proline-rich proteins bind calcium and phosphate and bring them to the apatite surface. Buffering Bicarbonate and, to a lesser extent, phosphate and macromolecules help neutralize plaque pH after meals, thereby reducing time available for demineralization. Antimicrobial action Specific (sIgA) and non specific (lactoferrin, lysozyme and sailo peroxidase) help to control antimicrobial mechanisms, Digestion and taste Digestive enzymes found in saliva include amylase, protease, lipase and nuclease, water of saliva functions as a solvent to facilitate taste (Frederick and Rosen, 2001).

Saliva can be considered as one of the chief defense systems against aqueous soluble components of tobacco (Zappacosta *et al*., 2002). As formerly reported, some components of the saliva could neutralize the toxic effect of aldehydes, oxidants, and carcinogens. Saliva contains nonenzymatic and enzymatic antioxidant systems, chiefly superoxide dismutase (SOD), glutathione peroxidase, uric acid, catalase, and glutathione (GSH) (Moore *et al.*, 1994).

1.6 Calcium, Phosphate, Magnesium, and Tobacco Consumption Risk

There was strong epidemiological evidence showed linkage of tobacco consumption (smoking) with increased salivary calcium (Sewon *et al*., 2004; Kiss *et al*., 2010). Increased salivary calcium considered as risk factor for periodontal diseases .Almost all tobacco consumers have increased salivary calcium (Hegmann, 2012).

Regarding to tobacco consumption risk factor, there is high prevalence of tobacco consumption among youngest more than elderly, tobacco use almost always begins by the time kids graduate from high school before age of 18, also tobacco use among aged youth or adults (Samsha, 2001).

Other study stated that, significant reduced serum calcium level in the exposed group (smokers) due to direct interaction between cigarette materials including nicotine and calcium receptors which lead to impaired intestinal calcium absorption and consequently reduced calcium levels (Facuci *et al*., 2008).

The previous study showed that, significant increased in serum phosphate level in tobacco consumers when compared with non tobacco consumers (Pannuru *et al.,* 2009).

Several reports have indicated that, there is significant increased in serum magnesium level in smokers in comparison with non-smokers (Abdalla *et al*., 2013).

1.7 Rationale

Tobacco consumption is one of the most serious problems worldwide, and there is strong association between tobacco consumption and cancer (lung and gum), also cardiovascular diseases, hypertension and influence electrolyte balance (calcium) which is play major role in metabolism that is give reason to be a target for researchers to find out the early diagnosis and prevention, but there are no such studies in Sudan. Therefore this is the first study to determine the effect of Tobacco consumption on serum and saliva (calcium, phosphate, and magnesium) in Sudanese Tobacco consumers in order to develop future prevention strategies for the tobacco consumption in this country.

1.8 Objectives

1.8.1 General Objective

To evaluate the effects of tobacco consumption on serum and saliva calcium, phosphate and magnesium in Khartoum State

1.8.2 Specific Objectives

1. To estimate the concentration of calcium, phosphate, and magnesium in the saliva and serum in the study population.

2. To compare these concentrations between tobacco consumer populations.

3. To compare these concentrations of tobacco consumers, with the ones of tobacco non-consumers.

4. To correlate between serum and salivary calcium, phosphate and magnesium concentration and duration of tobacco consumption, on the one hand, and quantity of daily tobacco consumption on the other hand.

2 Materials and Methods

2.1 Materials

2.1.1 Study Design

This is descriptive cross-sectional study to estimate serum and saliva calcium, phosphate and magnesium in Sudanese tobacco consumers as test groups and Sudanese non-tobacco consumers as control group.

2.1.2 Study Area

The study was conducted in Sudanese population in Khartoum state during the period from February 2014 to June 2014.

2.1.3 Study Population

The targeting group in this study is Sudanese of tobacco consumption (males) as test groups and Sudanese non-tobacco consumption (males) as control group.

2.1.4 Inclusion Criteria

Test group; Sudanese of tobacco consumption (smoking –snuffing).

2.1.5 Exclusion Criteria

Others types of tobacco consumption had been excluded.

2.1.6 Sampling

Sudanese healthy106 males 76 tobacco consumption as test group (30smokers, 25 snuffers, and 21 both (smoking and snuffing) and 30 non- tobacco consumption as control group were enrolled in this study.

We did randomized survey and selection of the subjects who comply with our inclusion criteria. Any subject complying with those inclusion criteria, and who is present the day of data collection was systematically taken into account.

The blood samples (4 ml) were taken in the morning through superficial venous puncture on dry tubes .Saliva (3 ml) was also collected in the morning.

The blood samples collected were centrifuged at 3000 RPM during five (5) minutes in order to get serums. The latter will be used the same day to determine calcium, phosphate and magnesium concentration. Saliva samples collected were also centrifuged at 3000 RPM during five (5) minutes, and supernatant fluid was used to determine calcium, phosphate and magnesium concentration.

2.1.7 Data collection

A questionnaire was administered to each selected subject. Blood sample was drawn and saliva collected from each subject, complying with the inclusion criteria.

2.1.8 Ethical consideration

Before the study conducted the proposal of the study were ethically approved by ethical committee of the Sudan University of Science and Technology. Then the verbal informed consent obtained from volunteers.

2.2. Methods

2.2.1 Calcium Estimation

2.2.1.1 Principle

Calcium in the sample reacts with methylthymol blue in alkaline medium forming a colored complex that can be measured by spectrophotometry. hydroxyquinoline is included in the reagent to avoid magnesium interference.

2.2.1.2 Procedure

1. Conditions were adjusted: wave length (570nm), cuvette (1cm light path), temperature ($37C^{O}$/15-$25C^{O}$).
2. The instrument was adjusted to zero with working reagent.
3. 1ml from working reagent which composed of reagent A (potassium cyanide, ethanolamine) and reagent B (methylthymol blue, hydrochloric acid, hydroxyquinoline) were pipette into blank, standard, and sample tube.
4. 10µl from standard was pipette to standard tube, and 10µl from sample to labeled samples tubes.
5. Then tubes were mixed well and incubated the tubes for 2 minutes at room temperature.
6. The absorption (A) of the standard and the sample was read at 570nm against the blank. The color is stable for at least 1 hour.

2.2.1.3 Calculation

The concentrations of Serum and saliva were calculated according to the following formula:

(A) Sample (A) standard x 10(standard conc.) in mg/dl.

2.2.1.4 Reference Value

8.6 - 10.3 mg/dl**.**

2.2.1.5 Sensitivity

30Ma.dl/mg =120mA.L/mmol

2.2.1.6 Linearity

15mg/dl calcium = 3.75 mmol/L calcium. For higher values dilute sample1/2 with distilled water and repeat measurement.

2.2.2 Magnesium estimation

2.2.2.1 Principle

Magnesium ions react with xylidyl blue in alkaline medium to form a water soluble purple-red cheiate, the color density of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexion with EDTA.

2.2.2.2 Procedure

1. All reagents and samples were allowed to come to room temperature (18-25 C^{o}).
2. One ml of color reagent was pipette in reagent blank tube, standard tube and labeled samples tubes.
3. 10µl of (distilled water, standard and sample) was pipette in reagent blank tube, standard tube and labeled samples tubes respectively.
4. Then tubes were mixed well and incubated for 5 minute at room temperature.
5. The final absorbance of the sample and standard was measured at 520nm against the reagent blank.

2.2.2.3 Calculation

The concentrations of Serum and saliva were calculated according to the following formula:
(A) sample/(A) standard x 2(standard conc.) in mg/dl.

2.2.2.4 Linearity

The linearly of this method is to 2.00 mmol/l (4.9mg/dl). Samples with higher concentration should be diluted 1+1 with distilled water and reassayed. Multiply by 2.

2.2.2.5 Sensitivity

The minimum detectable level has been determined as 0.07mmol/l.

2.2.2.6 Reference value

1.7- 2.7 mg/dl.

2.2.3 Phosphorus Estimation

2.2.3.1 Principle

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry.

2.2.3.2 Procedure

1. Conditions were adjusted: wavelength (340nm), cuvette (1cmlight path), temperature $37C^{O}$.
2. The instrument was adjusted to zero with working reagent.
3. 1ml from working reagent which composed of (sulfuric acid, sodium chloride, and ammonium molybdate) were pipette into blank, standard, and labeled samples tubes.

4. 10µl from standard was pipette to standard tube, and 10µl from sample to labeled samples tubes.

5. Then tubes were mixed well and incubated for 5 minutes.

6. The absorption of the sample and standard was read against the blank solution.

2.2.3.3 Calculation

The concentrations of serum and saliva were calculated according to the following formula:

(A) sample/(A)standard x 5 (standard conc.) in mg/dl.

2.2.3.4 Reference Value

Adult: 2.5 - 4.5 mg/dl

Children 4 – 7 mg/dl

2.2.3.5 Linearity

20 mg/dl =6.46 mmol/l.

2.2.4 Statistical analysis

The data was analyzed using SPSS computer program, frequency, mean; STD, and cross tabulation were calculated and ANOVA test used.

3 Results

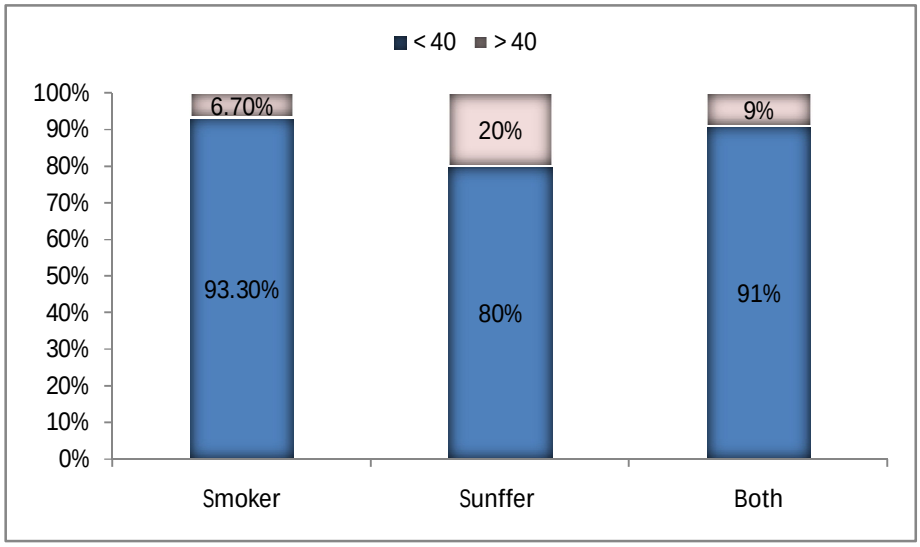

Fig (3.1) Showed distribution of tobacco consumption in different age group, expressed as percentage (%)

Table (3.1) Comparison the mean of serum and saliva Calcium, Phosphate, and Magnesium between Tobacco types (cases) and Control.

	Case (76)	Control (30)	*P*-value
Serum Calcium	6.49 ± 1.94	8.55 ± 1.3	0.000
Saliva Calcium	10.57 ± 2.57	6.03 ± 2.07	0.000
Serum Phosphate	5.2 ± 1.15	4.9 ± 1.38	0.300
Saliva Phosphate	16.67 ± 4.6	16.44 ± 4.6	0.080
Serum Magnesium	1.71 ± 0.44	1.56 ± 0.33	0.090
Saliva Magnesium	1.03 ± 0.35	0.99 ± 0.29	0.500

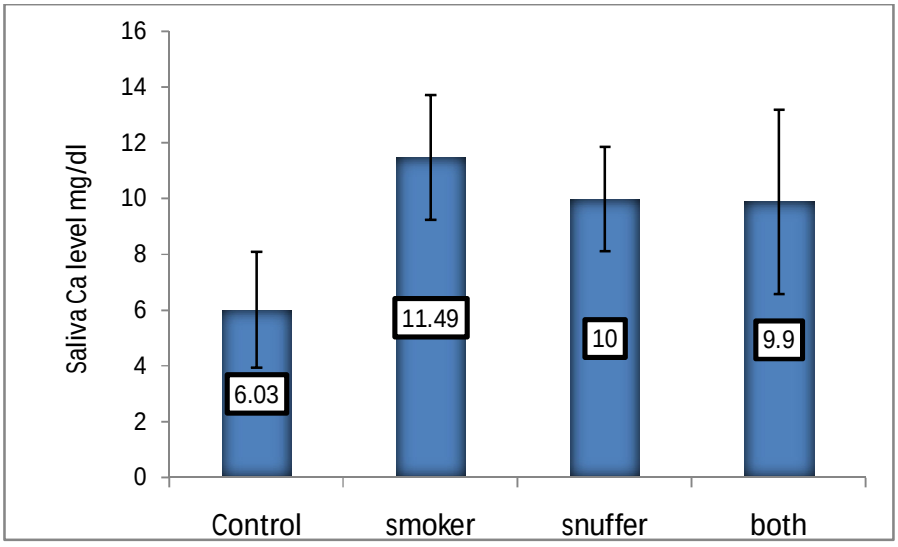

Fig (3.2) Showed mean concentration of saliva calcium mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value < 0.05)

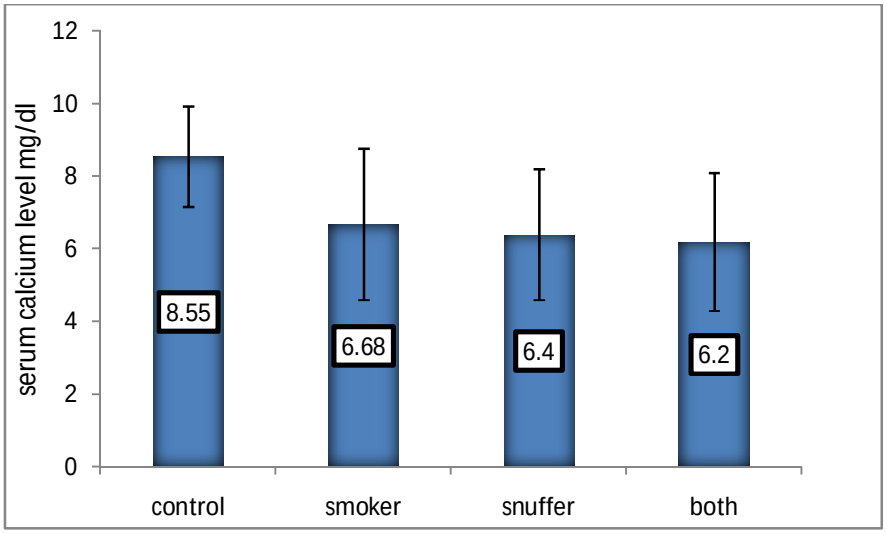

Fig (3.3) Showed mean concentration of serum calcium mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value < 0.05)

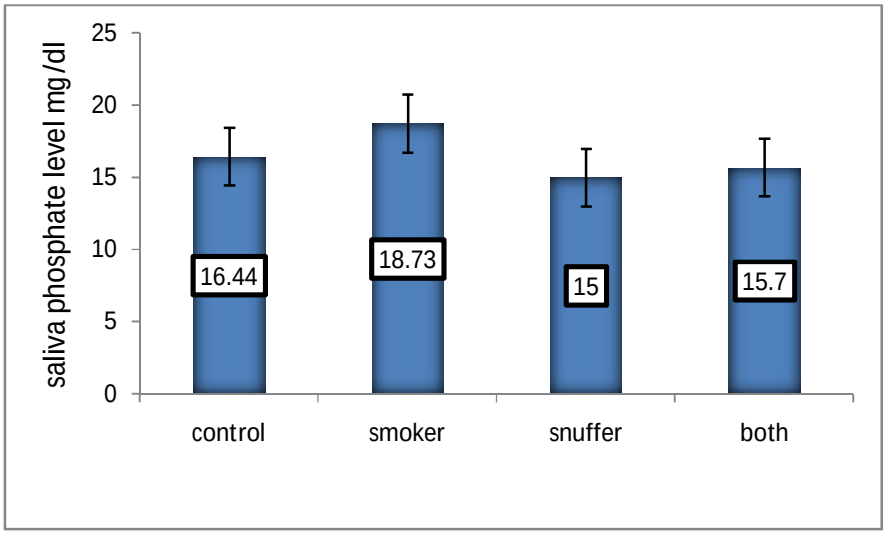

Fig (3.4) Showed mean concentration of saliva phosphate mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value 0.08

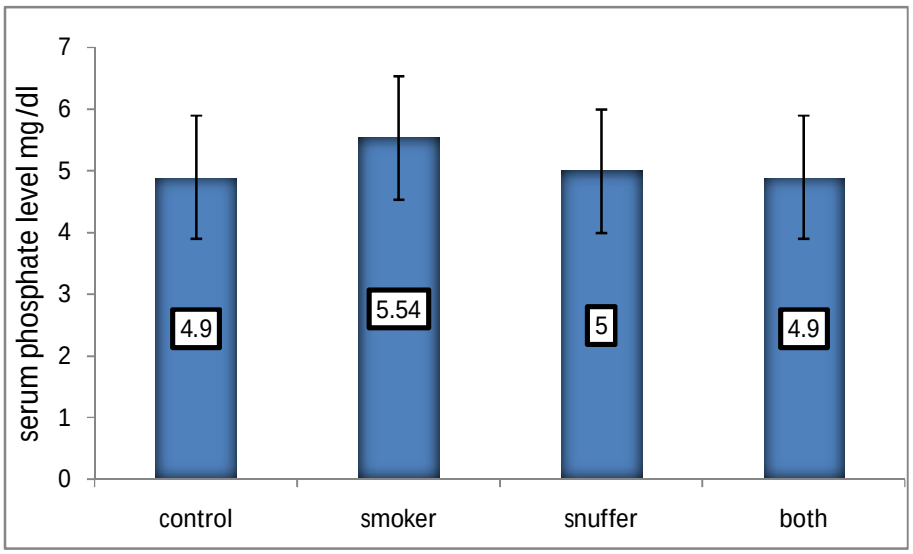

Fig (3.5) Showed mean concentration of serum phosphate mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value 0.3

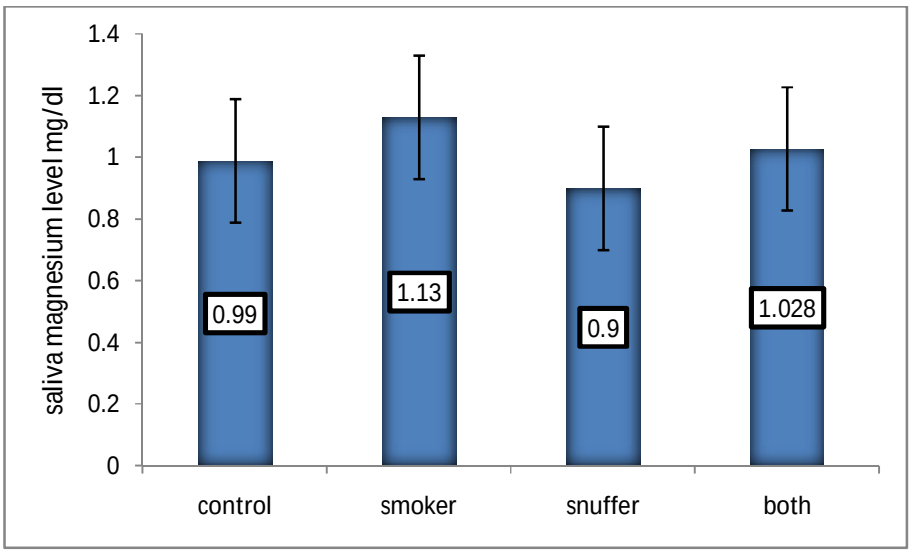

Fig (3.6) Showed mean concentration of saliva magnesium mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value 0.5

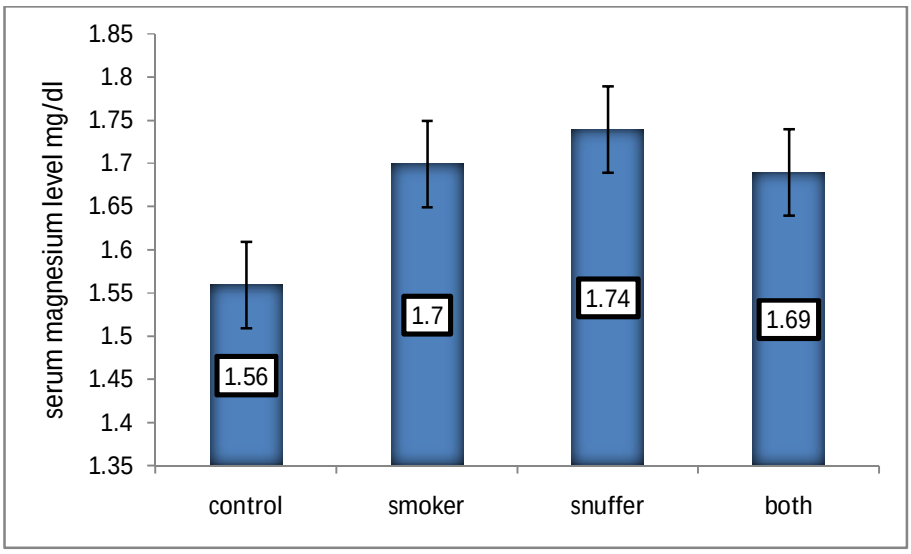

Fig (3.7) Showed mean concentration of serum magnesium mg/dl in smoker, snuffer, both and control group, result expressed as (M ± SD) and *P*-value 0.09

4 Discussions, Conclusion and Recommendations

4.1 Discussions

Tobacco consumption (smoking) is world-wide major cause of preventable morbidity and mortality. Smokers are at greater risk for cardiovascular diseases, cancer, peptic ulcer, and gastroesophageal reflux, bone matrix loss and hepatotoxicity. Researchers observed that, smoking causes alterations in calcium and electrolyte metabolism. Electrolyte disturbance may lead to life threatening metabolic abnormalities such as coronary heart disease and liver disease. Accordingly the present study aims to evaluate serum and saliva calcium, phosphate and magnesium in tobacco consumers and non tobacco consumers in Khartoum state.

The results of frequency showed that, the percentage of tobacco consumption among youngest males (less than 40years old) is greater than elderly (more than 40years old), in all groups of study (smokers, snuffers and both), account for (93.3%, 80% and 91 %) for youngest compared with (6.7%, 20% and 9%) in elderly. Several reports have indicated that smoking and tobacco use almost always begins by the time kids graduate from high school before age of 18, also tobacco use among aged youth or adults must include a focus on reducing experimentation and regular tobacco use among teenagers and pre-teens (Samsha, 2001).

Therefore our study observed starting smoking earlier, increase risk factor of smoking, this observations confirmed by Hegmann who reported an earlier age of smoking initiation means that the potential duration of smoking throughout a person life span is increased; therefore that person risk of developing lung cancer and demineralization of bone (Hegmann, 2012).

The results of the present study provide experimental evidence that there is significant increased in salivary calcium levels in study group when compared with control group resulting in (*P*-value 0.000) these finding agreed with proven study done by Ivana who stated that, there was strong epidemiological evidence showed linkage of tobacco consumption (smoking) with increased salivary calcium (Sewon *et al*., 2004; Kiss *et al*., 2010). In contrast serum calcium level showed significant decreased in study group when compared with control group, with (*P*-value 0.000) this finding similar to the previous study done by Facui who stated that, significant reduced serum calcium level in the exposed group (smokers) due to direct interaction between cigarette materials including nicotine and calcium receptors which lead to impaired intestinal calcium absorption and consequently reduced calcium levels (Facuci *et al*., 2008).

The present study showed insignificant difference in salivary phosphate level in study group when compared with control group with (*P*-value 0.08), in addition to insignificant difference

in serum phosphate level in study group when compared with control group with (*P*-value 0.3), these explained tobacco consumption has no effect in both salivary and serum phosphate level, these finding inverse to the previous study done by Pannuru who stated that, significant increased in serum phosphate level in study group when compared with control group (Pannuru *et al.,* 2009).

Several reports have indicated that, there is significant increased in serum magnesium level in smokers in comparison with non-smokers (Abdalla *et al*., 2013). Thus our study examined magnesium levels are found no significant difference in both serum and salivary magnesium level in study group when compared with control group with (*P*-value in 0.5), neither domestic nor international yet been conducted to study the effect of smoking in salivary magnesium levels.

The present study showed that insignificant difference in salivary calcium level in all study groups (smokers, snuffers and both) when compared with the control group with (*P*-value 0.3, 0.4 and 0.6) respectively, In addition to insignificant difference in serum calcium level in all groups of the study (smokers, snuffers and both) when compared with the control group with (*P*-value 0.5, 0.1 and 0.2) respectively.

Our study showed that insignificant difference in salivary phosphate level in all study groups (smokers, snuffers and both) when compared with the control group with (*P*-value 0.5, 0.04 and 0.7) respectively, In addition to insignificant difference in serum phosphate level in all groups of the study (smokers, snuffers and both) when compared with the control group with (*P*-value 0.05, 0.1 and 0.6) respectively.

The results of present study revealed that, insignificant difference in salivary magnesium level in all study groups (smokers, snuffers and both) when compared with the control group with (*P*-value 0.2, 0.8 and 0.3) respectively, In addition to insignificant difference in serum magnesium level in all groups of the study (smokers, snuffers and both) when compared with the control group with (*P*-value 0.5, 0.4 and 0.6) respectively.

Also the result of serum and salivary (calcium, phosphate and magnesium) showed no correlation between them and duration of tobacco consumption, as well as the age of study population.

4.2 Conclusion

- The study concluded that, Saliva calcium increases in tobacco consumers, more than non tobacco consumers, but there is a decrease in serum calcium in tobacco consumers when compared with the control group.
- There is no difference in the mean of salivary and serum (phosphate and magnesium) level between tobacco consumers and control group.
- There is no correlation between concentrations of (calcium, phosphate and magnesium) and ages of the study population as well as the duration of tobacco consumption.

4.3 Recommendations

- More research should be performed among large number of tobacco consumers to determine the correlation between (calcium, phosphate and magnesium) level and tobacco consumption. In addition to other parameters related to bone mineralization (PTH, ionized calcium, vitamin D) with emphasized to study local tobacco types (snuffers).
- Awareness by the effect of chronic smoking as risk factor for periodontal diseases and cause of calcium teeth demineralization.
- More advices to tobacco consumers and explain the complications of tobacco consumption.

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Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Level of Serum and Salivary Calcium - phosphorus - Magnesium in Habitual Adult Tobacco Consumers

Name: ..... No: .....

Age /Years: ..... Sex: .....

Residence: ..... Education Status: .....

Any Diseases: .....

Any Drugs used: .....

Type of Tobacco:

Smoking: Yeas: ..... No: .....

Tobacco: Yeas: ..... No: .....

Both (Smoking and Tobacco): Yeas: ..... No: .....

Duration of Consumption: .....

NO of Cigarette /day: .....

Times of tobacco /day: .....

Laboratory investigation:

Serum Ca^{++}: ..... mg/dl

Saliva Ca^{++}: ..... mg/dl

Serum Mg^{++}: ..... mg/dl

Saliva Mg^{++}: ..... mg/dl

Serum Ph^{-3}: ..... mg/dl

Saliva Ph^{-3}: ..... mg/dl