REVIEW OF LITERATURE
Tumor cells, just as other living cells, possess the potential for proliferation, differentiation, cell cycle arrest, and apoptosis. There is a specific metabolic phenotype associated with each of these conditions, characterized by the production of both energy and special substrates necessary for the cells to function in that particular state (25). Tumors are often some distance from capillaries and the cells within the tumor exist in an environment of low oxygen concentration (hypoxia). Therefore, most cancer cells derive their energy from anaerobic glycolysis, which means they must consume much more glucose for ATP production than a normal cell using oxygen to oxidize glucose. A cancer cell consumes glucose in amounts 4-5 times greater than normal cells. It has been found that cancer cells demonstrate altered metabolism when compared to normal cells. Weber (26) observed that cancer cells exhibit increased rate of pentose phosphate pathway activity that is characterized by an increase in glucose-6-phosphate dehydrogenase.

In the early part of 20th century, Otto Warburg (27) originated a hypothesis that the cause of cancer is primarily a defect in energy metabolism. He suggested that cancer was a metabolic disease in which respiration was damaged and anaerobic fermentation was increased resulting in a malignant phenotype. Cancer cells show clear difference in energy metabolism when compared to normal cells. Since the days of Warburg, studies have continued to show alteration of functional energy metabolism in cancer cells. Among these differences are: an increased rate of glycolysis, shifts in LDH isozyme patients. There is no standard treatment of recurrent cervical cancer that has spread beyond the confines of a radiation and surgical field. The results of the present work may be helpful in the early diagnosis as well as prognosis of the cervical cancer and may add in designing more fruitful treatment strategy.

Tumor formation has a way of triggering the body into a constant state of gluconeogenesis. Weber suggested that cancer cells exhibit increased rate of pentose phosphate pathway activity that is characterized by an increase in glucose 6-phosphate dehydrogenase. This way at no time is the tumor not acquiring the vast amounts of glucose it needs for growth. At the same time the tumor is inducing gluconeogenesis for its own energy supply, it is also taking critical elements from the normal healthy cells is what eventually leads to cancer cachexia (or the wasting syndrome). One of the most common
signatures of highly malignant tumors is their capacity to metabolize more glucose to lactic acid than their tissues of origin.

The glycolytic enzymes are present in large excess. The activities of the following enzymes are studied in normal, precancerous and malignant specimens from the human cervix: hexokinase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase and glucose 6-phosphate dehydrogenase. In precancerous conditions, only pyruvate kinase showed moderate but significant activity increases. In invasive carcinomas of the cervix, all the enzymes studied showed 2-4 fold increase as compared to the normal cervix (28). In intact cells glycolysis is limited by the availability of inorganic phosphate, which in turn is required for the regeneration of glycolytic ATP. Since phosphate transport is slow compared to glycolysis, the regeneration of inorganic phosphate and its availability for glyceraldehyde 3-p dehydrogenase is therefore in the final analysis the rate limiting factor of glycolysis. Rapidly growing cells that convert ATP to ADP in the course many synthetic processes can be expected to express their glycolytic capacity more effectively than cells with restricted synthetic functions. Hepatomas exhibiting this phenotype are dependent on the high expression of type II hexokinase which supplies such tumors with abundant amounts of glucose 6-phosphate, a significant carbon and energy source especially under hypoxic conditions. It is well known fact that if a tumor is more differentiated (the more rapidly it grows), the higher its capacity to carry out aerobic glycolysis and showed detectable mitochondrially associated hexokinase activity. In case of the slow growing tumors that are known to have a near liver capacity for aerobic glycolysis and don't have detectable mitochondrial hexokinase activity. The decreased amounts of oxygen within the tumor it does not undergo a complete cycle and increases lactic acid as a waste product into the blood stream (Lactic acid is also the waste product of muscle metabolism. Lactic acid begins to form when sufficient oxygen supply is not being met and the burning sensation occurs). Because the tumor cell does not utilize oxygen as a normal cell, it gives off constant quantities of lactic acid. This increased elevation of lactic acid in the blood stream can be correlated with the rate of tumor growth. The tumor uses its own lactic acid production to recycle through the liver to be converted back into glucose. This is often called a 'metabolic circuit'.
Carbohydrates serve as the primary source of energy in the cell and carbohydrate metabolism is central to all metabolic processes, which can accomplished either anaerobically or aerobically, resulting in the synthesis of ATP. After the formation of glucose-6-phosphate, the major pathways of glucose metabolism include glycolysis and pentose phosphate cycle. Glycolysis results in the formation of NADPH (29). Pyruvate is an important junction point in glucose metabolism and a substrate for the formation of acetyl CoA, an entry point to the TCA cycle and mitochondrial oxidative phosphorylation (30). Pyruvate kinase, regulating the exit of the glycolytic pathway, determines the relative amount of glucose that is channeled into the synthetic processes or used for the glycolytic ATP production (31-35). As early as 1904, it was shown that $\text{H}_2\text{O}_2$ causes a rapid nonenzymatic and stoichiometric decarboxylation of pyruvate to acetic acid, $\text{H}_2\text{O}$ and CO$_2$. It was recently discovered that mammalian cells pyruvate as an antioxidant defense to O$_2$ radicals, and other hydroperoxides. NADPH, besides being the source of reducing equivalents for the glutathione/glutathione peroxidase/glutathione reductase system, has also been shown to participate in the metabolic decomposition of $\text{H}_2\text{O}_2$ and organic hydroperoxides (36). Therefore, in addition to the well known role of glucose metabolism in energy production, it appears to be involved with cellular sensitivity to oxidative stress mediated by hydroperoxides as by products of oxidative metabolism presumably via the formation of pyruvate and NADPH. It has been shown that increasing glucose concentrations in the tissue culture media render CHO cells resistant to $\text{H}_2\text{O}_2$ induced cytotoxicity. Recently, it has been discovered that simply removing glucose from the cell culture medium (glucose deprivation) induces cytotoxicity and oxidative stress in human tumor cells.

In 1931, Warburg first discovered that cancer cells have a fundamentally different energy metabolism compared to healthy cells. Malignant tumors frequently exhibit an increase in anaerobic glycolysis – a process whereby glucose is used as a fuel by cancer cells with lactic acid as an anaerobic by product compared to normal tissues. The large amount of lactic acid produced by this fermentation of glucose from cancer cells is then transported to liver. This conversion of glucose to lactate generates a lower, more acidic pH in cancerous tissues as well as overall fatigue from lactic acid build up. This inefficient
pathway for energy metabolism yields only 2 moles of ATP energy per mole of glucose, compared to 38 moles of ATP in the complete aerobic oxidation of glucose (37, 38).

The inhibition of glycolysis by oxygen (Pasteur effect) in normal cells is largely a result of allosteric inhibition of phosphofructokinase by ATP, whose generation depends on the presence of oxygen. This inhibitory action of ATP can be reversed by increased by levels of fructose 1,6 bisphosphate (39). The activity of phosphofructokinase affects the other two key glycolytic enzymes, i.e., hexokinase and pyruvate kinase. First, utilization of fructose 6-phosphate leads to a decrease in the cellular levels of glucose 6-phosphate. Glucose 6-phosphate is a potent inhibitor of hexokinase, resulting in hexokinase activation (40). Secondly, the activation of phosphofructokinase elevates the levels of fructose1,6 bisphosphate, which even in micromolar concentrations activates pyruvate kinase (41).

The glycolysis of erythrocytes represents an ideal experimental basis for a unique theoretical treatment for several reasons:

(a) glycolysis is practically uncontaminated by any other interfering pathway; neither glycogenolysis nor gluconeogenesis need to be considered, and even the oxidative pentose pathway is insignificant.

(b) There are no internal compartments in the cell.

(c) The data on enzyme kinetics and metabolic concentration are superior to those of most other cell.

(d) Finally a number of external effectors influence in a profound and reproducible manner.

The mature mammalian erythrocyte, for all its physiological importance, has one of the least complicated biochemical organizations of the body cell types. It has no nucleus, endoplasmic reticulum or mitochondria, and it therefore unable to synthesize nucleic acids or proteins and lacks a Krebs cycle and electron transport system. The erythrocyte is composed of two independent units: the membrane and the cytoplasm. The cytoplasm contains hemoglobin as a major element and is equipped with all he enzymes of glycolysis. The mature red cell depends entirely on the Embden-Meyerhof pathway for its energy. Here
glucose is metabolized to lactic acid generating a net of two ATP molecules per molecule of glucose consumed. Glucose is also metabolized via the Pentose phosphate pathway which generates reducing power in the form of NADPH. Although in most other tissues NADPH is a source of energy, does not appear to be energy-linked in the mature red cell. Despite its low energy production, the red cell can maintain itself for approximately 120 days. During this time it is subjected to numerous physical and chemical insults. The end result of these factors is probably the slow depletion of enzyme and cofactors which cannot be replaced by the red cell.

Most cancer cells exhibit increased glycolysis and use this metabolic pathway for generation of ATP as a main source of their energy supply. This phenomenon is known as the Warburg effect and is considered as one of the most fundamental metabolic alterations during malignant transformation. In recent years, there are significant progresses in our understanding of the underlying mechanisms and the potential therapeutic implications. Biochemical and molecular studies suggest several possible mechanisms by which this metabolic alteration may evolve during cancer development. These mechanisms include mitochondrial defects and malfunction, adaptation to hypoxic tumor microenvironment, oncogenic signaling, and abnormal expression of metabolic enzymes. Importantly, the increased dependence of cancer cells on glycolytic pathway for ATP generation provides a biochemical basis for the design of therapeutic strategies to preferentially kill cancer cells by pharmacological inhibition of glycolysis. Several small molecules have emerged that exhibit promising anticancer activity in vitro and in vivo, as single agent or in combination with other therapeutic modalities. The glycolytic inhibitors are particularly effective against cancer cells with mitochondrial defects or under hypoxic conditions, which are frequently associated with cellular resistance to conventional anticancer drugs and radiation therapy. Because increased aerobic glycolysis is commonly seen in a wide spectrum of human cancers and hypoxia is present in most tumor microenvironment, development of novel glycolytic inhibitors as a new class of anticancer agents is likely to have broad therapeutic applications.

It was observed that tumor cells have increased steady state levels of $O_2^-$ and $H_2O_2$ associated with aberrant respiration, which causes damage and inactivate signaling
pathway leading to malignant phenotype. Therefore, it appears that tumor cells have an aberrant respiration, which is compensated by an increase in glycolysis and pentose phosphate cycle activity as a compensatory mechanism to protect themselves from an increase in the steady state level of \( H_2O_2 \). Glucose metabolism has been shown to be involved with cellular sensitivity to oxidative mediated by hydroperoxide, presumably via the formation of pyruvate and NADPH. Since mitochondrial metabolism would be the preferred route of energy production during glucose deprivation, it is reasonable to hypothesize that glucose deprivation would create a metabolic state where \( O_2^- \) and \( H_2O_2 \) concentration were increased and scavenging via pyruvate as well NADPH dependent reactions was decreased. This would be expected to result in a condition of metabolic oxidative stress characterized by increased levels of pro-oxidant production and increased levels of oxidized glutathione. The effects are not as noticeable at first, but as tumor progresses and uses more and more glucose the effects become much more obvious.

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred as prooxidants. On the other hand compound and reactions deposing these species, scavenging them, suppressing their formation or opposing their actions are antioxidants. In normal cells, there is an appropriate pro-oxidant: antioxidant balance. However this balance can be shifted towards the pro-oxidant when production of oxygen species is increased greatly or when levels of antioxidants are diminished. This state is called “Oxidative stress” and can result in serious cell damage if the stress is massive or prolonged.

There is strong evidence that \( O_2^- \) itself does not interact with lipids, \( O_2^- \) reacts with lipid peroxides as well as \( H_2O_2 \) in metal ion catalyzed Haber Weiss reaction (42) to produce toxic hydroxyl radical (HO•H) which could account for initiation of LPO (lipid peroxidation) by formation of lipid radicals (L•) and lipid peroxy radicals (LOO•). The LPO process is the manifestation of any kind of membrane modification, which has been revealed by membrane fluidity study. Membrane fluidity is an important barrier of inter and intracellular communication, membrane elasticity and biological transport of proteins and lipids. Any change in the level of unsaturation of the phospholipids fatty acyl chain, cholesterol to phospholipids ratios and fatty acyl chain length (43) influence membrane
fluidity. Higher saturated fatty acid to unsaturated fatty acid ratio is indicative of decreased membrane fluidity. Levin et.al (44) proposed that oxidation of membrane lipids results in the formation of peroxidation degradation products (e.g. Malondialdehyde) which lead to the cross-linking reaction of the lipid-lipid and lipid-protein type thereby rendering the membrane more rigidity and less fluidity. Peroxidation process is an important indication of membrane damage, which serves a lot to promote irreversible dysfunction of essential cellular components and ultimately triggers accidental cell death or necrosis (45).

Reactive oxygen metabolites (ROMs), including superoxide anion (O$_2^*$-), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (*OH), play an important role in carcinogenesis. There are some primary antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which protect against cellular and molecular damage caused by the ROMs (46).

Reactive oxygen species (ROS), represented by superoxide, hydrogen peroxide and hydroxyl radicals, have been implicated in many diseases including cancer (47). ROS have been known to play an important role in the initiation and promotion of multistep carcinogenesis. The cellular antioxidants play a crucial role in protection against neoplastic disease. However, very little is known about the antioxidant defense in cervical carcinoma. This is addressed in the present study.