

## Research Article

# Role of mitochondria in progression of cancer: a semi-quantitative study

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## ABSTRACT

Mitochondria have been an area of scientific study for more than 100 years. It was in early 20<sup>th</sup> century that Otto Warburg first described differences in the mitochondria of tumors v/s normal cells. It was observed that tumor cells have increased rate of aerobic glycolysis compared with normal cells. The study was carried out in patients diagnosed as premalignant and malignant conditions which had three objectives that is to demonstrate the presence or absence of mitochondria in cytological smears, in order to perform a semi-quantitative analysis on the number of mitochondria and to identify the difference in distribution mitochondria if any. The study was carried out in the Department of Oral and Maxillofacial Pathology of S.P.D.C., Sawangi, Wardha with consent from patients and approval from the institutional ethical committee. 20 patients each diagnosed clinically and histo-pathologically with OSCC and Premalignant conditions or lesions respectively were selected for the purpose of the study. 20 subjects who had come for routine endodontic treatment were taken as control group for the purpose of the study. It was observed that there was even distribution of mitochondria throughout the cytoplasm in smear that had been taken from normal mucosa which appeared sharply defined whereas in premalignant mitochondria were located in the perinuclear zone and 10% in the peripheral zone and in malignant conditions distribution was sparse in the perinuclear area and appeared ill-defined.

**Keywords:** Mitochondria, Premalignant, Malignant, Warburg Effect, Altmann stain, Cancer

## INTRODUCTION

In cell biology, mitochondria is a membrane enclosed organelle found in most eukaryotic cells. These organelles range from 0.5 to 10 micrometers ( $\mu\text{m}$ ) in diameter. Mitochondria are described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. Mitochondria are also involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth.<sup>1</sup>

Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction and may play a role in the aging process. A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins.

Disruption of the outer membrane permits proteins in the inter membrane space to leak into the cytosol, leading to certain cell death. Cytochrome C is present in inter membrane space. The inner mitochondrial membrane contains proteins with five types of functions.

1. Those that perform the redox reactions of oxidative phosphorylation
2. ATP synthetase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery.
5. Mitochondria fusion and fission protein

The inner mitochondrial membrane has the ability to produce ATP. The matrix contains a highly-concentrated mixture of hundreds of enzymes, special mitochondrial

ribosomes, tRNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include synthesis of ATP, oxidation of pyruvate and fatty acids, and the citric acid cycle.

Mitochondria have been an area of scientific study for more than 100 years. It was in early 20<sup>th</sup> century that Otto Warburg first described differences in the mitochondria of tumors v/s normal cells. It was observed that tumor cells have increased rate of aerobic glycolysis compared with normal cells.

This increase was due to impairment of the respiratory capacity of tumor cells. The physical structure, composition and function differ greatly from that of tumor and normal cells.<sup>2</sup>

The mitochondria of many rapidly growing tumors are fewer in number, smaller and have fewer cristae than that of slowly growing tumors. Polypeptide profiles and lipid composition of the inner mitochondrial membrane of tumor cells differ from those of normal cells. Additional differences have been described with regard to the substrates, mitochondrial membrane potential, rate of electron transfer, anion transfer, protein synthesis, organelle turnover and reactive oxygen species.<sup>3</sup>

Mitochondria are key players in several cellular functions including growth, division, energy metabolism, and apoptosis. The mitochondrial network undergoes constant remodeling and these morphological changes are of direct relevance for the role of this organelle in cell physiology. Mitochondrial dysfunction contributes to a number of human disorders and may aid in cancer progression. Prominent features of cancer cells include metabolic imbalances and enhanced resistance to mitochondrial apoptosis.

In 1955, Otto Warburg, recipient of the 1931 Nobel Prize for Medicine or Physiology, attributed cancer to damage the mitochondria, which are tiny structures within each cell that are involved in energy production, that is the manufacture of ATP.

The fact that tumors rely heavily on glycolysis to meet their metabolic demands has been recognized since the beginning of the twentieth century, yet a complete elucidation of the Warburg effect has not been achieved.

According to Warburg, due to the irreversible damage to the mitochondria tumour cells shifted from respiration to fermentation, a much less efficient method for producing ATP. This particular aspect had been evaluated from cytological smears by staining with vital stains in the present study. Also the feasibility of its use in diagnosis of pre-malignant and malignant conditions is evaluated.<sup>5</sup>

#### **Aim and Objectives**

The study was carried out in patients diagnosed as premalignant and malignant conditions which had the following three objectives

- (a) To demonstrate the presence or absence of mitochondria in cytological smears.
- (b) To perform a semi-quantitative analysis on the number of mitochondria.
- (c) To identify the difference in distribution mitochondria if any.

#### **METHODS**

This study was carried out in the Department of Oral and Maxillofacial Pathology of S.P.D.C., Sawangi, Wardha with consent from patients and approval from the institutional ethical committee. 20 patients each diagnosed clinically and histo-pathologically with OSCC and Premalignant conditions or lesions respectively were selected for the purpose of the study. 20 subjects who had come for routine endodontic treatment were taken as control group for the purpose of the study.

Deep smears were taken from normal, premalignant conditions or lesions and OSCC individuals for cytological smear preparation. Vital staining was carried out by using Altmann's technique. Mitochondria present in the cytoplasm of cell seen in the smears obtained from normal, premalignant conditions or lesions and OSCC individuals respectively were visualized under light microscope at 40x magnification.

#### **Procedure for Altmann Staining**

Scrape was taken by wooden stick and transferred on glass slide.

↓

Fixed in Helly's fluid for 24 hours

↓

Washed overnight in running water

↓

Slide was dried except where the sections were present

↓

It was flooded with acid fuchsin in aniline-water, and heated gently until 'steaming', in order to over stain.

↓

Acid fuchsin was washed off with distilled water

↓

The section was observed under the high power of the light microscope, and irrigated with an alkaline solution.

↓

One drop of saturated aqueous sodium carbonate solution in 10 cc of distilled water gave a fairly rapid differentiation, taking 30 sec. to 1 minute.

↓

When the slide had been correctly differentiated, it was washed in distilled water, then counter stained progressively in a 5 per cent, aqueous solution of water-soluble methyl blue.

↓

Mitochondria appeared as pink dots that were irregularly distributed in the cytoplasm of cells seen in smears of normal, premalignant and malignant conditions when visualized under light microscope at 40x magnification

**RESULTS**

It was observed that there was marked difference in the distribution of mitochondria in malignant, premalignant and normal conditions. Changes in the number and distribution of mitochondria were demonstrated using semi-quantitative method.

In the present study, we had divided a cell into 2 areas using a circle made half way between nucleus and outer cell membrane dividing the cell into

- (A) Peripheral zone
- (B) Perinuclear zone

This was done for the ease in assessment in the distribution of mitochondria.

The appearance of mitochondria on staining with Altmann’s technique:-

**Normal Control**

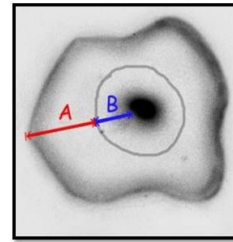
There was even distribution of mitochondria throughout the cytoplasm in smear that had been taken from normal mucosa which appeared sharply defined.

**Premalignant Conditions**

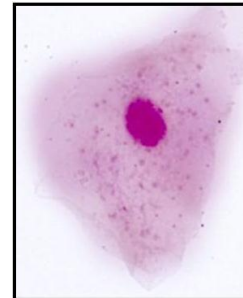
Whereas, in case of premalignant conditions the distribution of mitochondria was predominantly in perinuclear area and was indistinct in places. 90% of mitochondria were located in the perinuclear zone and 10% in the peripheral zone.

**Malignant Conditions**

In case of malignant conditions the distribution was sparse in the perinuclear area and appeared ill-defined.



**Figure 1: A cell into 2 areas using a circle made half way between nucleus and outer cell membrane dividing the cell into: (A) Peripheral zone (B) Perinuclear zone.**



**Figure 2: The appearance of Mitochondri and staining with Altmann's technique:- Normal cell.**



**Figure 3: The appearance of Mitochondri on staining with Altmann's technique:- Malignant Cell.**

**Table 1: Following conditions were drawn from the above observations.**

Condition	Demonstration	Characteristic	Distribution	Inference
Normal	Sharp	Abundant	Evenly distributed throughout the cytoplasm	+++++
Premalignant	Indistinct	Reduced	90%perinuclear area and 10% in peripheral area	+++
Malignant	Ill-defined	Scanty	Sparse and limited to the perinuclear area	+

**DISCUSSION**

Cytological smears had been obtained from malignant, pre-malignant and normal patients. Three distinct characteristics that had been observed in the cell cytoplasm are:-

- (a) The change in the number of mitochondria.
- (b) The change in the distribution of mitochondria.
- (c) The change in the size and shape of mitochondria.

Reasons for variation of no. and distribution of mitochondria in normal, pre-malignant and malignant

cells. Decrease in the number and change in distribution of mitochondria was observed from normal to premalignant to malignant cells this decrease occurred due to:

- (1) Change in cytoplasmic pH of cell.
- (2) Shift in the signaling pathway

Warburg hypothesized that cancer, malignant growth, and tumor growth are caused by the fact that tumor cells mainly generate energy by non-oxidative breakdown of glucose. This is in contrast to "healthy" cells which mainly generate energy from oxidative breakdown of pyruvate.<sup>6</sup>

For proliferating tumor cells to function, aerobic glycolysis is therefore a necessary adaptation to cope with the lack of ATP generation by oxidative phosphorylation. Such adaptation results in an acidic microenvironment that is caused by excess lactate production.<sup>7</sup>

Thus, cancer cells tend to synthesize ATP mainly through 'aerobic glycolysis', a metabolic state that is linked to high glucose uptake and local acidification owing to lactate production. Due to this there was shift of equilibrium making the cytoplasm acidic and causing changes in cell membrane potential and normal expression of cell into abnormal.

This leads to an acidic pH in the cytoplasm of a cell making the survival of the mitochondria in the cell difficult. This reason can be attributed as the reason for the decrease in number of mitochondria. Also, the progressive acidification in the cytoplasm of cell from periphery to centre that is from the cell membrane to the nucleus could be the reason for variation in the distribution of mitochondria.<sup>8</sup>

Though, survey of literature could not provide an explanation for this. We hypothesize that as the cell changes from normal to premalignant to malignant; there is a change in membrane potential of cell cytoplasm that causes alteration in mitochondrial outer membrane potential making the environment unfavorable for the survival of mitochondria in them.<sup>9</sup>

Therefore, this resulted in gradual reduction in the distribution of mitochondria in the cytoplasm from the cell membrane towards the nucleus. Hence, we hypothesize that as the condition progresses from pre-malignant to malignant the distribution of mitochondria gradually decreases from periphery of the cell towards the nucleus.<sup>10</sup>

Reasons for variation of shape and size of mitochondria in normal, pre-malignant and malignant cells:-

There was significant alteration that was associated with the change in shape and size of mitochondria was due to:

- (1) Mitochondrial DNA mutation
- (2) Alteration in the MOMP of cell.

Mitochondria are involved either directly or indirectly in many aspects of altered metabolism in cancer cells. Their shapes vary from small spherical fragments in rapidly dividing cells to elaborate branched networks in quiescent cells. Various tumor cell lines exhibit differences in the number, size and shape of their mitochondria relative to normal controls.<sup>11</sup>

Mitochondrial DNA has been proposed to be involved in carcinogenesis because of high susceptibility to mutations and limited repair mechanisms in comparison to nuclear DNA. In majority of cases, mitochondrial mutations were multiple, implying possible accumulation of mtDNA damage. Which has been attributed as one of the causative factors alteration in shape and size.<sup>12</sup>

Altmann's staining technique in cytological smears for diagnosis of pre-malignant and malignant conditions:-

The cytological smear preparation is simple to conduct and can be carried out on daily basis with minimum skill and equipment required. It can act as an important diagnostic marker in detection of risk associated pre-malignant conditions as well as in known conditions as another confirmatory tool alongside histo-pathological diagnosis.

Altmann's method is one of the most specific stain for mitochondria. It is a stain that individually only stains the mitochondria amongst the cell organelles. The stain tends to have an affinity for mitochondrial structure making the mitochondrial membrane permeable leading to its demonstration in the cytoplasm of cell.

It does not require any special training for its conduction neither is it time consuming and also is economic. Also, this stain can be used for staining non-keratinized cells that are obtained in the scrape which cannot be identified by PAP staining technique.<sup>13</sup>

## CONCLUSION

Mitochondria are dynamic intracellular organelles that play a central role in oxidative metabolism and apoptosis. The recent resurgence of interest in the study of mitochondria has been fuelled in large part by the recognition that metabolic alterations in this organelle are causative or contributing factors in a variety of human diseases including cancer.

Several distinct differences between the mitochondria of normal cells and cancer cells have already been observed at the metabolic level. As documented in this study, distinct difference in number and distribution of mitochondria were observed in controls and compared to premalignant and malignant conditions.

There was a marked decrease in the number of mitochondria observed in the smears that had been obtained from normal to pre-malignant to malignant. Similarly variation in the distribution of mitochondria was also observed, from generalized throughout the cytoplasm to being restricted mainly to the perinuclear area from normal to pre-malignant to malignant. This resulted in marked difference between normal, premalignant and malignant, therefore this staining technique can provide important insight to distinguish between them and serve as clinical tool in high risk cases.

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