Abstract
Gastric carcinoma, in India, is the second most prevalent cause of cancer-related death since most patients are asymptomatic until the disease progresses to advanced stages. Hence, there is a need for non-invasive and specific biomarkers for early screening and diagnosis. Human mitochondrial DNA (mtDNA) has 37 genes involved in oxidative phosphorylation pathway (OxPhos). There are several 100 to 1000 mitochondria in a human cell and each mitochondrion has two to 10 copies of mtDNA. There is a significant association between the mtDNA copy number and an increase in risk of various cancers. There is also a relation between the changes in the sequence of mtDNA in genes, such as MT-CYB, MT-ATP6 and gastric cancer, according to which the tumor cells switch to aerobic glycolysis for ATP production even in the presence of oxygen due to Warburg effect. Multiple factors have an adverse effect on mitochondrial gene expression and impairs the OxPhos pathway due to lack of sophisticated DNA repair mechanism in mitochondria. Techniques, such as Next Generation Sequencing and Whole Genome Sequencing, are capable of early detection of copy number variants and mtDNA mutations in blood sample essential for better prognosis of gastric cancer. Through the course of this study, various reports of a correlation between mtDNA damage and gastric cancer were analyzed and it was found that the increasing evidence of the role of mtDNA and its copy number in cancer indicates its significance as a potential biomarker for GC.

Keywords: Gastric Cancer, Mitochondrial DNA, Mutation

Introduction to Gastric Cancer
Gastric adenocarcinoma is the most common type of malignancy in the stomach and comprises 90% of all the gastric cancers. It is usually caused by environmental factors and accumulation of specific genetic alterations. There are three main causes for gastric cancer.
Helicobacter pylori (H. pylori): H. pylori induced gastric cancer is due to the progression of chronic gastritis into malignancy. It is the most prevalent cause of gastric cancer. This is due to the increase in the production of pro-inflammatory proteins, such as interleukins and TNF, possibly induced due to diverse genetic alterations.

Mutations: CDH1 gene mutation coding for E-Cadherin is associated with both sporadic and familial cases of gastric cancer. Methylation of the promoter region of this gene drastically reduces the gene expression and the loss of E-Cadherin function plays a key role in the development of diffuse gastric cancer.

EPV: 10% of gastric adenocarcinoma is associated with Epstein–Barr virus. These generally have a diffuse morphology and occur in the proximal stomach. Biopsies have revealed that the tumor is developed by the multiplication of one infected cell. EBV infection may occur later in gastric carcinogenesis.

Epidemiology
Over one million reports of gastric carcinoma are identified per year globally. Gastric cancer is the 7th most prevalent cancer worldwide and the 5th most commonly diagnosed. It is more prevalent in males than females with the ratio of male to female diagnosis varying from 1.83 to 2.2 based on the development of the country. Stomach cancer is more commonly diagnosed in developed countries. Incidence can differ from 6.6 to 20 per 100,000 males and this variation is due to factors, such as region and culture. The rate of incidence is the highest in Eastern and Central Asia and Latin America and the lowest in North and East Africa. Cancer-associated risk pertaining to gastric carcinoma is 8.3%. Regarding India, there is a wide regional variation observed in the prevalence of gastric cancer with some states having incidence rates as high as several individual countries. From the available data, the states of Mizoram and Tamil Nadu have the highest incidences in males and Bengaluru for females while Gujarat has the lowest incidence of gastric carcinoma. India has shown a steady increase in the reports of gastric cancer from 2015 to 2020 and the number of cases diagnosed in 2020 is estimated to be 50,000 cases.

Types of Gastric Cancer
The Lauren classification divides gastric cancer into Intestinal and Diffuse type based on the different patterns of molecular alterations.

Diffuse type is composed of discohesive cells with an infiltrative growth pattern. The cells have a characteristic signet ring cell morphology owing to the mucin vacuoles pushing nucleus to periphery. These tumors impart a “leather bottle” appearance termed linitis as they evoke a desmoplastic reaction stiffening the gastric wall and possibly triggering diffuse rugal flattening and a rigid, thickened plastica. The diffuse type lacks intercellular adhesion due to the diffuse invasion growth pattern of cells throughout the stroma. It is more common in the younger population.

In Intestinal type, the cells are bulky, composed of glandular structures, and form either an exophytic mass or an ulcerated tumor along the broad cohesive fronts. The tumor cells often contain apical music vacuoles. It can be correlated to lifestyle choices, such as smoking, alcohol consumption, unhealthy diet, and Pylori infection. It is more prevalent in elderly people.

Altered Cellular Metabolism – Warburg Effect
Altered cellular metabolism is an important hallmark of neoplastic cells, allowing their uncontrolled proliferation. Even in the
presence of excess oxygen, these cells take up high amounts of glucose which is then converted to lactic acid through the glycolytic pathway in a process called fermentation. This is alternatively known as aerobic glycolysis or Warburg effect (Figure 1) after Otto Warburg, the Nobel Prize winner in 1931 for this discovery. Following the discovery of this theory, the metabolism of cancer cells has been an active area of research.\(^1\) Positron Emission Tomography is applied to scan tumor cells by injecting patients with f-fluorodeoxyglucose, a glucose derivative that is preferentially taken up by tumor cells.\(^2\)

However, the question arises of why cancer cells prefer aerobic glycolysis to oxidative phosphorylation when the former only gives 2 molecules of ATP while the latter produces 36 ATP molecules.\(^3\) This is because Warburg metabolism provides the cells with the metabolic intermediates, namely acetyl-CoA and nucleic acids, required for the synthesis of cellular components essential for proliferating cells quickly. These intermediates also supply the pentose phosphate pathway (PPP) required for cancer development.\(^4\) Various signaling pathways downstream of growth factor receptors are deregulated via mutations in oncogenes and tumor suppressor genes causing metabolic reprogramming in neoplastic cells. For example, ATP Synthase is essential for mitochondrial OxPhos and the dysfunction of this enzyme leads to Warburg effect.\(^5\)

Thus, the function of mitochondria in tumor cell metabolism is not to generate ATP, but to carry out the reactions generating metabolic intermediates which can be utilized as precursors for biomolecule synthesis. Therefore, the dysfunction of mitochondria is important for aerobic glycolysis and occurs due to the mutations in genes, such as MT-CO1 and UCP1.\(^6\)

Mitochondrial DNA

Human mitochondrial DNA (mtDNA) is a double stranded, circular DNA, accounting for only 0.0005% of the nuclear genome.\(^7\) It comprises 37 genes which code for 2 rRNAs, 22 tRNAs, and mRNAs for 13 polypeptides of the oxidative phosphorylation (OxPhos) system. It also contains approximately 1.1 kb of non-coding DNA (D-loop) comprising sequences important for the initiation of mitochondrial replication and transcription. The length of the strand in humans is approximately 16,569 bp.\(^8\) There are several 100 to 1000 mitochondria in a human cell and each mitochondrion has two to 10 copies of mtDNA depending on cellular energy demand. mtDNA is known to be maternally inherited, yet the biparental transmission is sometimes observed. An example of this can be seen in a study by Luo et al., in which the biparental transmission was found in three separate multi generation families.\(^9\)

The strands are distinguished based on the nucleotide composition. Heavy strand (H-strand) is guanine rich whereas the light strand (L-strand) is cytosine rich.\(^10\) The genetic code differs from nDNA. The stop codons are AGA and AGG while UGA codes for tryptophan. At the post transcriptional level, to compensate, UAA codon must be introduced. The AUA codon coding for isoleucine in nDNA encodes for methionine in mtDNA.\(^11\) There are a few diseases associated with mtDNA mutation, such as MELAS, LOHN, Pearson's bone-marrow–pancreas syndrome, Kearns–Sayre syndrome (KSS), and Myoclonus epilepsy with ragged red fibres (MERRF).

mtDNA Alterations in Gastric Cancer

Several studies have indicated that 65% of the examined gastric cancer patients have at least one somatic mtDNA mutation,\(^12\) such as large-scale insertions and deletions, point
mutations and copy number variations. In a study by Rui Bi et al., in the four gastric cancer tissues, seven somatic mutations were identified. Two mutations (m.2148 2149dupAG and m.3200T>C) were in the MT-RNR2 gene, one mutation (m.9770T>C) was synonymous while four mutations (m.8572G>A in the MT-ATP6 gene, m.15777G>A and m.15597T>C in the MT-CYB gene, and m.4632G>A in the MT-ND2 gene) were found to be non-synonymous. In another study performed by Jiang et al., eight out of 10 patients with gastric cancer had mtDNA mutations and the missense mutations were frequent. Wu et al observed that 90% of the gastric cancers had mtDNA mutations, including point mutations, tandem duplication, triplication in the D-loop, 4,977-bp deletion, and mtDNA depletion.

Copy Number Variation of mtDNA and its Correlation to GC
Multiple studies have demonstrated the correlation between mtDNA copy number variants and the behavior of malignant cells, such as cell growth, metastasis, and drug sensitivity. There has been an observed positive association of elevated risk of gastric cancer with an increase in the amount of mtDNA in peripheral blood. However, as the stage of gastric cancer advances from well-defined cells in stage I and II to poorly differentiated cells in stage III and IV, the mtDNA content in cells decreases due to the demethylation of D-loop. This reduction is a late molecular event brought about by the excessive production of ROS in mitochondria during the progression of gastric cancer. The change in the amount of mtDNA found in peripheral blood is consistent with Warburg effect.

D-Loop of mtDNA
The D-Loop region of mtDNA contains major regulatory sites for transcription and replication. Hence, it is the most frequent site of somatic mutation in cancer with the incidence ranging from 4% to 48%. The results in a study done by Wu et al showed that the somatic mutations in the D-loop of the mtDNA was observed in several gastric carcinomas during carcinogenesis. It is considered as a mutational hotspot with capability to modify the activity of mitochondria and mtDNA. In four gastric cancers, high levels of a 50-bp deletion with a 9-bp direct repeat at D-loop region were reported. Approximately 13% of the examined gastric cancers had a 260-bp tandem duplication/triplication in the D-loop region of mtDNA. Mononucleotide repeat variants of the poly-C sequence was the most common mutation observed in mtDNA.

Oxidative Damage
The mutation rate of mtDNA is much higher than that of nDNA in spite of DNA repair mechanisms, namely base excision repair, which is due to the proximity of mitochondrial ROS production to the mtDNA. ROS is produced as a consequence of normal aerobic respiration creating a highly oxidative environment causing DNA damage. The enhanced mitochondrial oxidative stress has a direct relation with the oxidative DNA damage and mtDNA mutagenesis. Zhou et al indicated that there were 18 mutations associated with a rise in ROS production, apoptosis, and proliferation in patients with gastric carcinoma.

There are various defense mechanisms against oxidative stress to maintain mitochondrial integrity. Primary mechanisms include protective molecules or proteins scavenging ROS and secondary mechanisms involving enzymes that repair...
the damage. However, these mechanisms are not as sophisticated as the nuclear DNA mechanisms. If these stress defense mechanisms are compromised or overwhelmed, it leads to mutation in mtDNA.41

**mtDNA Polymerase Damage**

mtDNA is replicated and repaired by the nuclear encoded DNA polymerase gamma (PolG), which is a target of oxidative damage42 and a frequent site of mutations in cancerous tissue.43 The mutations were identified in all the three domains of PolG protein, exonuclease domain, linker region, and the polymerase domain.44 Increased mtDNA mutations have also been observed in PolG\(^{\text{exo-}}\) and PolG\(^{\text{exo+/-}}\) mice.45, 46 Hence, the generation of mtDNA mutations and genome instability in cancer may be enhanced if defects in the polymerase and repair activities of POLG exist.36

**H. pylori Infection**

*H. pylori* is a gram-negative, microaerophilic bacterium associated with various stomach related infections. It is able to survive the harsh acidic environment of the stomach due to its ability to convert urea to ammonia, thereby neutralizing the environment.47 It has also been known to be the main risk factor for cancers, such as gastric and colorectal. Thus, it has been identified as a type 1 carcinogen by the World Health Organization.48, 49 Several studies have implied that *H. pylori* infection can impair mitochondrial DNA function and repair the mechanisms, such as nDNA mismatch repair (MMR), thus inducing genetic instability in gastric cells. It has been associated with the oxidative DNA damage in the D-loop and the genes encoding proteins of the Electron Transport Chain, thereby disrupting OxPhos. APE-1 and YB-1 are the multifunctional proteins which play a major role in Base Excision Repair (BER) and possible mitochondrial targets for *H. pylori*.34 Thus, in *H. pylori* infections, the frequency of mutations in mtDNA is increased, copy number and OxPhos capacity are decreased, and DNA repair mechanisms, namely BER and MMR are impacted. All the changes are linked to the progression of gastric cancer and can be identified through PCR.51

**Defects in Proteins Controlling mtDNA Biogenesis**

The decrease in mtDNA copy number can also be linked to the defect or downregulation in proteins localized in the mitochondria, such as p53, SIRT3, POLG, and PGC-1. These proteins control DNA replication and maintenance along with mitochondrial biogenesis. For example, p53 which is confined to mitochondria, interacts with POLG and helps maintain mtDNA stability; therefore, the loss of p53 leads to the decreased copy number of mtDNA.52 SIRT3 is known to be downregulated in gastric cancer and since it is a mitochondrial deacetylase, it is associated with the decreased mtDNA integrity and copy number.53 PGC-1 expression is commonly linked to increased mitochondria production and consequent poor tumor advancement in various cancer types. The defect in this protein thus causes decreased mtDNA content.54, 55 There are various proteins whose dysfunction leads to the progression of gastric cancer due to mitochondrial damage.36

**Detection Methods**

The most common method to quantify and study mtDNA in peripheral blood is quantitative PCR, employed to monitor mtDNA copy number variants in real time.56 The earlier norm was PCR followed by downstream processing methods, such as electrophoresis, to analyze specific mutations and more recent methods, such as
DNA microarray techniques and Sanger sequencing. However, the technological advancements over the last 10 years have led to the development of newer high throughput screening methods of DNA detection, such as Next Generation Sequencing (NGS).\(^5\)\(^7\), \(^5\)\(^8\), \(^5\)\(^9\) NGS helps in analyzing cancer genome profiles through specifically sequencing the target genes for a wide variety of genes using techniques, such as Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS), and RNA Sequencing (RNA-Seq).\(^6\) Not only do these methods of DNA analysis have higher resolution compared with the traditional methods, but also have been proven to help examine the unexplored genomic regions, thereby giving us a chance to better understand the function of these components.\(^6\), \(^6\)\(^1\) Thus, the use of high throughput screening methods, such as NGS in the detection of mtDNA shows great promise in interpreting gastric tumorigenesis.\(^6\)\(^2\)

### Other Diagnostic Measures

To date, the standard method for the early detection and screening of pre-malignant lesions and cancer progression is endoscopy followed by a biopsy. This involves the use of an endoscope to investigate the interior of hollow organs of the body and the examination of cellular morphology by applying H and E staining. Histopathological studies have been considered as the gold standard for diagnosis.\(^6\)\(^3\) Additionally, TNM staging is determined utilizing CT, PET, and other imaging modalities.\(^6\)\(^4\) Most Asian countries also turn to photofluorography for detection. Another technique employed is sentinel node mapping for the early stage detection of gastric cancer. SN mapping is commonly used in melanoma and breast cancer, but recently it has also been used for gastric cancer. Circular and microRNAs can also be employed as novel biomarkers in the detection of gastric cancer.\(^6\)\(^5\), \(^6\)\(^6\), \(^6\)\(^7\)

### Conclusion

Mitochondria are important organelles in a cell since they perform various functions, such as ATP generation and biosynthesis of macromolecules, which are essential for cellular proliferation and growth. Mitochondria have their own genome apart from the nuclear DNA in a cell and defects in this DNA are seen in cancer progression. There have been reports of mutations advantageous to neoplastic cells in the mitochondrial genes regulating numerous downstream pathways. An increase in mtDNA copy number is also observed in the patients with the elevated risk of gastric cancer. It is then seen to decrease as the gastric carcinoma progresses to more severe stages. The reason behind this decrease is the demethylation of D-loop which is the most common site of mutation in mtDNA. This change is in accordance with Warburg effect, assisting the growth and development of tumor cells by producing metabolic intermediates required for biomolecule synthesis.\(^2\)\(^0\) The close proximity of mtDNA to ROS and the defects in mtDNA polymerase and proteins controlling biogenesis are some of the key factors that lead to mtDNA alterations mentioned above. Another important risk factor in GC progression is \(H.\) \(pylori\) infection. Knowledge of these changes in mtDNA is helpful in developing new high throughput detection methods, such as NGS, which are sensitive to mutated mitochondrial genes. Therefore, mtDNA is a potential biomarker for early diagnosis of gastric cancer.\(^6\)\(^8\)

### Conflict of Interest

None declared.

### References

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Figure 1. This image illustrates the cellular mechanisms depicting Warburg effect in neoplastic cells. Even in the presence of oxygen, cancer cells undergo aerobic glycolysis to produce intermediates, such as Acetyl CoA, to be supplied to the Pentose Phosphate Pathway for cancer development. This can be linked to mtDNA mutations in genes, such as mtCO1 and UCP1.

Acetyl CoA: Acetyl Coenzyme A; TCA cycle: Tri Carboxylic Acidic cycle; FADH: reduced Flavin Adenine Dinucleotide; NADH: reduced Nicotinamide Adenine Dinucleotide; ETC: Electron Transport Chain; mtCO1: mitochondrially encoded Cytochrome C Oxidase 1; UCP1: Uncoupling Protein 1; ROS: Reactive Oxygen Species.