# Evolution Mitochondrial tRNA$^{trp}$ Encoded Gene Variation in Colorectal Cancer

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*Abstract*—**Back ground: Colorectal cancer (CRC) is a significant disease in Iraq that increased in the last years, the role of Transfer RNA (tRNA) types in cell functions still under investigations in different disease, tRNA level, genetic variation, and its derivative molecules were studied.**
**Objective: the present study aims to evaluate mitochondrial tRNA$^{trp}$ encoded gene variation in colorectal cancer in a case control design.**
**Methodology: about 48 CRC patients with 30 healthy individuals were enrolled in the present study, PCR –sequencing method was used to read the whole tRNA$^{trp}$ gene sequence, variations were detection according to NCBI SNPs.**
**Results: The results showed that the patient group has two types of CRC, adenocarcinoma was high frequent (80%) than Mucinus adenocarcinoma (20%), four stages; the III stage was more observed than others (54.28%), and according to the grade, the well differentiation was more seen (57.14%) than moderate and poor differentiation. The genetic analysis showed a significant association of tRNA$^{trp}$ total genetic variation with CRC (p <0.0014), about 15 SNPs were detected in the patients group and 1 SNP in the control group, non-significant association of SNPs with CRC. Conclusion: according to present findings, the variation of tRNA$^{trp}$ encoded may be associated with CRC.**

*Keywords:* **tRNA$^{trp}$ , mitochondrial encoded gene, variation, colorectal cancer**

## INTRODUCTION

The structure and modifications in Transfer RNA (tRNA) sequences' have a vital role in its function. In the nucleus through tRNA generation the precursors have transcribed pre-tRNAs by RNA polyIII and transcription factors TFIIIB and TFIIIC, then it modified and exported to the cytoplasm (Santos et al., 2019). Berg and Brandl, (2021) elucidated that The modified tRNA after transcription to form mature tRNA via put aside the 5′leader by RNase P, La protein linked to the U tract of the 3′ end, and Rnase Z splite the discriminator nucleotide, The several tRNA structure consist of an acceptor stem and D-arm, that discriminate aminoacyl tRNA synthetase, the anticodon arm for choosing correct amino acid, the T-arm assist in ribosome interaction; and the variable loop (Lyons et al., 2018). In spite of the basic structure of tRNA, the anticodon and acceptor stems may be differentiated in tRNA types, this variations make tRNA molecules to be flexible for its interaction with others molecules in the cell (Kuhn, 2016), then modifying enzymes may make modifications to the mature tRNA, (Santos et al., 2019). Berg and Brandl, (2021) found About 93 tRNA modifications, in eukaryotes Pan, (2018) observed about thirteen modifications per tRNA. The primary functions of tRNA modification are stabilized the tertiary structure, influence cell signaling pathways, the codon-anticodon recognition and the cellular response to stress (Lyons et al., 2018).

The CRC is the most common disease among cancer types in the world, with high rates of incidence and mortality in adults have age lower than 50 years (Favoriti et al., 2016; Bray et al., 2018; Ahmed , 2020). Regarding to the WHO, at the 2018 about (1.8) million of CRC new cases were reported worldwide and (862,000) deaths, and the global burden is expected to balloon to (2.2) million new cases and (1.1) million deaths at 2030 (WHO, 2020).

Notably, the rapidly proliferation of tumor cells, the high growth rate needs cellular components. Despite of most likely more of an impact than a tumor proliferation causes, some cancer cells have high tRNAs levels (Gingold et al., 2014). As well as several tRNAs were up-regulated more than ten times in breast tumor cells (Pavon-Eternod et al., 2009). Evidence found that Oncogenes have a vital role in the high gene expression, by obstructing tumor suppressors capability to prevent RNA polyIII transcription in normal cells, oncogenes can elevate Pol III transcription which raised tRNA transcription (Bian et al., 2021). In addition to selectivity in induction, some tRNA types than others, like tRNA methionine has been found to be stimulated in the most proliferating cells, while the tRNA of selenocysteine has been found to be prevented in some proliferating cells (Hudson et al., 2012; Pavon-Eternod et al., 2013; Barroso et al., 2014; Luchman et al., 2014; Santos et al., 2019). The current study aims to evaluate the tRNA$^{trp}$ encoded gene variation in CRC patients.

## METHODOLOGY

### *Study Design*

A case control study was suggested to evaluate the tRNA$^{trp}$ variation which encoded by mDNA, 5512..5579, gene: TRNW", GeneID:4578,HGNC:HGNC:7501, MIM:590095.

### *Study Subjects*

Aout 48 cases were enrolled in the present study with 30 healthy individuals; the cases were attended the privet histopathology

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lab for diagnosis. All patients were diagnosed as CRC using macro and microscopic by prof. Dr. Liwaa Al-Kelaby (university of Kufa\ college of medicine) in addition to bio and clinical markers. The control group was apparently healthy, embedded tissue was used for DNA extraction from cases and blood samples were used for the control group due to the normal DNA copy all cell types in healthy individuals.

*Ethical Approval*

All contributors enrolled in the present study with written consents according to ethical approval of ministry of higher education and scientific research.

DNA extraction: DNA was extracted from embedded tissue and blood by DNA extraction kits purchased from favorgen company (http://www.favorgen.com/en/).

*Target DNA Amplification*

Modified nested PCR was used to amplify the target sequence, about 68 n of $tRNA^{trp}$ were amplified with flanked sequences (60 n) in both sides of the gene, nested –PCR was implemented more than one time to obtain a high concentration of amplicons. Then PCR products were sequenced by genetic analyzer (macrogen company).

*Exclusion And Inclusion Criteria*

The present study included CRC patients without viral infection, Diabetes mellitus, hypertension or autoimmune disease. All patients included in the present study were before chemotherapy or radiotherapy treatment.

*Data Analysis*

DNA sequence analysis were implemented using MAFFT https://mafft.cbrc.jp/alignment/server/, SNPs and nucleotide variation by NCBI database https://www.ncbi.nlm.nih.gov/gene/4578 , odd ratio (CI95%) used for significant detection of genetic variants of p value less than 0.05.

## RESULTS AND DISCUSSION

The present work included colorectal cancer patients which diagnoses by histological changes, well differentiation of malignant glands, infiltrate the sub mucosa and muscular layer of the bowel wall were observed (figure 1). the Patient group have age mean (56.45±2.29) years while control group age was (33.1±2.07) years and the female percentage in the patient group was (62.9%) and the male was (37.1%).

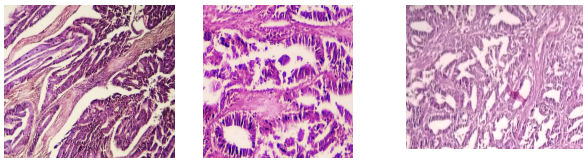

Figure (1) histo-pathological changes of CRC study group (H and E).

The present patient group characterized by two types of CRC, adenocarcinoma was high frequent (80%) than Mucinus adenocarcinoma (20%), the III stage was more observed than others (54.28%) and according to the grade, the well differentiation was more seen (57.14%) than moderate and poor differentiation (table 1).

TABLE (1) THE CRC PATIENT GROUP CHARACTERIZATION.

| CRC classification | Percentage (%) |
|---|---|
| Type of CRC | |
| Mucinus adeno ca | 20% |
| Adeno ca. | 80% |
| Stages | |
| I | 20% |
| II | 22.85% |
| III | 54.28% |
| IV | 2.85% |
| Grade | |
| Well differentiation | 57.14% |
| Moderate differentiation | 40.0% |
| Poor differentiation | 2.85% |

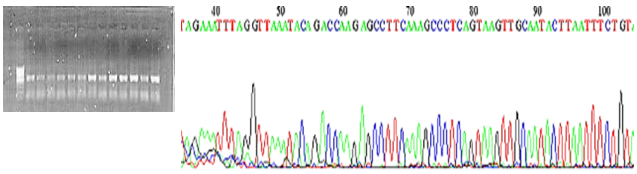

Figure (2) the electrophoresis pattern and DNA sequencing histogram of $tRNA^{trp}$ encoded gene in study groups.

The total mutation rate of $tRNA^{trp}$ in the CRC group was 1.34% while in the control group was (0.052%) in significant differences (OR 25.8628, CI95%3.5272 to 189.6341, p <0.0014) (figure 3).

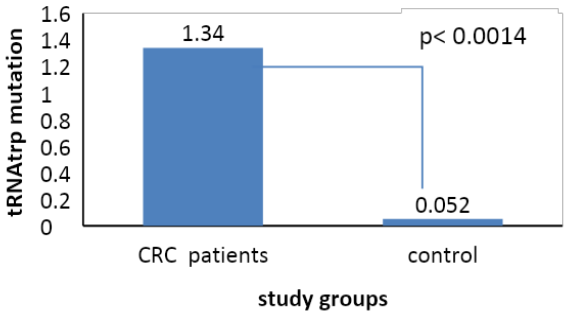


Figure (3) the total mutation rate of $tRNA^{trp}$ in study groups

About 15 variants in tRNA are clarified in table (2), results showed that the frequency of these variations were more observed in a CRC group than the control group in non-significant differences OR ranged (0.0545- 0.6226) at p values ranged (0.0890-0.7803).

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TABLE (2) SOME TRNA$^{TRP}$ VARIANTS IN STUDY GROUPS

| mDNA sites | Control | CRC Patients | Odd ratio | CI95% | P |
|---|---|---|---|---|---|
| A 689922 G | 1(3.57%) | 4(11.42%) | 0.0545 | 0.0019 to 1.5582 | 0.0890 |
| A 689923 G | 0 | 5(14.28%) | 0.6226 | 0.0223 to 17.3925 | 0.7803 |
| A 689924 G | 0 | 4(11.42%) | 0.4909 | 0.0172 to 14.0239 | 0.6774 |
| G 9556 A | 0 | 3(8.57%) | 0.3684 | 0.0124 to 10.9047 | 0.5635 |
| T 155882 G | 0 | 2(5.71%) | 0.2542 | 0.0081 to 8.0186 | 0.4367 |
| T 689925 C | 0 | 2(5.71%) | 0.2542 | 0.0081 to 8.0186 | 0.4367 |
| A 689926 G | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| T 689927 C | 0 | 2(5.71%) | 0.2542 | 0.0081 to 8.0186 | 0.4367 |
| rs1603220007 A>G | 0 | 2(5.71%) | 0.2542 | 0.0081 to 8.0186 | 0.4367 |
| A 689931 G | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| A 689929 T | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| G 689930 A | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| G 689933 A | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| C 9558 T | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |
| G 689943 A | 0 | 1(2.85%) | 0.1475 | 0.0040 to 5.3782 | 0.2969 |

The age mean of the patient group was more than 50 years, several studies deal with present finding and disagree with others, In Iraq Al-Saigh et al., (2019) suggested that the CRC is significant disease, With a high percentage of middle age. Ibrahem et al., (2022) found that CRC in Iraq is still a disease of the elderly and is elevated incidence and mortality. Belong to age, Farhad et al., (2023) found that 31.6% of CRC cases were below than 50 years of age. The CRC depending on the complex factors, age is one of them.

The association of tRNA encoded genetic variation with cancers still under investigations, the present study found the total mutation rate in tRNA$^{trp}$ significant association with CRC but the single nucleotide variation (SNPs) or mutation didn't associate with CRC, evidences have been proved that tRNA modification, structure, and regulation were changed according to cell type and response to cell conditions which could effect in cell proliferation. The way of extending tRNAs cleaved or modified can detect the cell survival. The tRNA function in the normal cells becomes more complex in cancer cells, studies proved that the tRNA derivatives have unique roles in cancers (Huang et al., 2018).

Different modifications were happened to adjust the tRNA translation rate to reach the cell's requirements (Hou et al., 2015). Theses modification included acetylation, hydroxylation, and deamination (Suzuki, 2021) which effected in the tRNA's default mode of reading codons via rules of base pairing by effecting the translation efficiency and precision, or certain tRNA types abundance (Endres et al., 2019). like, the N6-threonylcarbamoyladenosine ($t^6A$) tRNA modification, that generate by the residue adding to the $N^6$ site of adenine aides in codon discrimination, aminoacylation, and translocation, while queuosine, a derivative of hypermodified guanosine, at site 34 can effect on the translation elongation rate (Suzuki, 2021). Furthermore tRNA methyltransferase ALKBH8 perhaps impact in mRNA translation by stimulating the hydroxylation of $cm^5U$ or $mcm^5U$ into $chm^5U$ or $mchm^5U$, respectively, in tRNA$^{Gly}$ (U*CC), which linked to tumor cell progression, moreover upregulation of ALKBH8 was found in bladder cancer with elevation in ROS level (Fu et al., 2010). The low variation of tRNA in the present study may be because the several hypomodified tRNAs are degraded via tRNA decay process using exonucleases. However, not static remains of some hypomodified tRNAs or it dependents on cellular conditions (Santos et al., 2019; Suzuki, 2021). As well as tRNA guanine transglycosylase that change specific tRNAs to alternate guanine for queuine in differentiated somatic cells, it incompletely modifies undifferentiated quickly growing cells (Pathak et al., 2005).

In cancer cells, the abnormal cellular conditions were contributed in differentiate tRNA modifications. Belong to high tumor cells proliferation, the blood supply is insufficient to cancer cells that cause hypoxia and oxidative stress, which stimulate a multitude of tumor-activating signaling cascades that upregulate tRNA modifying enzymes, Lead to catalyze tRNA modification and increasing tRNA translation (Endres et al., 2019). This is proved by anticodon wobble uridine tRNA modifications are upregulated in different cancer types (Hawer et al., 2018). In spite of the depended of this modification on the cancer types, modifications of U34 were observed to support the shift in translation in tumor cells and growth enhancing (Hawer et al., 2018).

## CONCLUSION

according to the curtail role of tRNA in cell function and the possibility of tRNA modification role in uncontrolled tumor cell proliferation, the present study was suggested, the association of tRNA$^{trp}$ encoded gene variation in mitochondria with CRC was showing significant variation but non-significant associated with SNPs types. Further investigations may be clarified the main role of tRNA$^{trp}$ with the CRC

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characterization, like the concentration of $tRNA^{trp}$, tRNA modification enzymes levels and its genetic polymorphisms, and tRNA derivative molecules.

Limitation of study: the sample size was limited in the present study because most patients were attended after chemo or radiotherapy.

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