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Detection of Mitochondrial A12308G Mutation in tRNA$^{Leu(CUN)}$ Gene and it's Relation with Colorectal Cancer Risk

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Abstract: ***A mitochondria have important role in oxidative phosphorylation, each cell have many copies of mitochondria. Mitochondrial DNA (mtDNA) in human is a 16. 569 kb circular double stranded molecule able for replication, transcription and translation. MtDNA represent the unique genetic material outside the nucleus in eukaryotic cells. This study was conduct on 36 Iraqi patients with colorectal disease to analyze the genetic mutation in A12308G of mtDNA tRNA$^{Leu(CUN)}$ gene for its etiology role in inflammation bowel and colorectal tumorgenesis using conventional polymerase chain reaction (PCR) and sequencing analysis. The results referred to that total frequency of the A12308G transition was showed 6 variations out of 18 patients (33.3%) in colorectal patients but these differences were not statistically significant, similar distribution for the frequency of the G12372A transition mutations was found in combination with A12308G polymorphism patients .Total of 7 out 18 patients (38.8%) were presence as insertion (ins) base between (12413-12414). Statistically significant results with high rate of deletion 11924A base was showed in 10 out of 18 samples (55.5%), coexisted of A12308G with G12372A mutations in addition to that in combination of A12410 C with T12414A may have synergistic effect in functions and associated with risk factor in colorectal cancer, finally large deletion site in (12380...12425) and (12383...12425) positions were described only in two tumor patients may be effect the colorectal cancer progression. In conclusions: The mtDNA A12308G tRNA$^{Leu(CUN)}$ gene variant may be don't have risk factor for malignant process of colorectal. Identify the predictive role of the mt tRNA^{Leu}(CUN) gene variations may be use as genetic biomarker in colorectal cancer, high rate of novel mutations that were found may serve as supporting factor in colorectal etiology and tumorgensis.***

Keywords: Mitochondria, mtDNA , mt tRNA^{Leu}(CUN), colorectal, Iraq

1. Introduction

A mitochondrion is a small organelle surrounded by enclosed intracellular membrane in eukaryotic cells, it acts as a major energy source by supply adenosine triphosphate (ATP) via oxidative phosphorylation pathway and main source of free active oxygen species, responsible of apoptosis (1). It has function in cellular differentiation, cell signaling and control of the cell cycle in addition to cell growth (2). Mitochondria are able for all activity like DNA replication, RNA transcription, and translation using own genetic systems. Thousands copies of mt tRNA are found per cell, its number varies according to cell type. The genomic map of mammalian mitochondrial DNA (mtDNA)) is a 16.569-kb circular double stranded include two regions, the non coding region which regulates transcription and replication by containing the leading-strand origin of DNA replication and the main promoters for RNA transcription , the other coding region contains DNA sequence used to make a (12S) and (16S) ribosomal RNA genes and 13 protein coding genes (Cytb, ND1 to 8, Cox1 to 3, ATP6 and 8) encode subunits of the respiratory chain in addition to 22 transfer RNAs (tRNAs) are necessary for protein translation of mitochondrial DNA (3,4). Transfer RNA (tRNA) genes are important for the synthesis of new proteins, mutations or polymorphisms in tRNA genes have been considered a helpful biomarker for different tumors (5). The tRNA$^{Leu\ (CUN)}$ is the important gene that was most studies target in mitochonderial DNA variation, the 12308 site is situated in the tRNA$^{Leu\ (CUN)}$ gene which is encodes for the most represented amino acid in the mitochondrial respiratory chain, suggesting a key role of this tRNA in mtDNA-coded oxidative phosphorylation subunits (6).

There are many distinct pathways of colorectal cancer (CRC) etiology and development such as the chromosomal instability pathway, it is associated with the activation of K-ras mutations oncogenes and the inactivation p53 tumor suppressor gene (7,8). It's established that microsatellite instability genes pathway have a major role in dysfunction of other genes (9\az, 10). DNA methylation in specific gene promoters (such as *MLH*1 and O-6-methylguanine DNA methyltransferase (*MGMT*) genes) is the recent well-studied epigenetic pathway (11,12). Mitochondrial DNA was observed to be extremely mutated in colorectal cancer cells (13). Recent study highlighted on possible link between mtDNA A12308G mutation of tRNA$^{Leu(CUN)}$ and CRC (14).The main objective of this article was to analyze the genetic mutation in A12308G of mtDNA tRNA$^{Leu(CUN)}$ gene for its etiology role in inflammation bowel and colorectal tumorgenesis .

2. Materials and Methods

2.1 Tissue Samples

Thirty six patients with colorectal disease were enrolled in this study at the Endoscopy Department of Gastrointestinal Tract Hospital in Baghdad city between October 2014 and May 2015.The patients clinicopathologic data were obtained from the archive of hospital. This study was approved by the ethical committees of Baghdad University. Written informed approval for the publication of this report was obtained from all patients.

Analysis of the mtDNA A12308G alteration in tRNA$^{Leu(CUN)}$

Extraction of DNA from tissues was done using QIAamp DNA kit (QIAGENE) according to manufacturers. Convention polymerase chain reaction (PCR) was performed to amplified 521bp fragment to screen the A12308G mutation in mtDNA tRNA$^{Leu(CUN)}$. Sequences of primers set in the coding region, the first name ONP71 with sequence 5'TGCTAGTAACCACGTTCTCC-3' position L.F 11901-11920 5 'and the second name ONP46 with sequence 5' TTTGTTAGGGTTAACGAGGG-3' position H.R 12420-12401, according to Mohammed1 *et al*. report (14).

Each 25µl reaction mixture for mtDNA amplification include 12.5 µl of master mix and 1.5 µl of each primer and 9.5 µl of genomic DNA PCR amplifications were achieved in a Applied Biosystem 96 thermocycle. Thermocycling condition were 94°C for 5 min followed by 32 cycles of 94°C for 1 min, annealing temperature 50°C for 1min and 72°C for 45 s min and final extension 72°C for 10 min for 32 cycles. The PCR products were examined for specificity using 1.5 % agarose gel electrophoresis. All DNA templates were processed for direct sequencing of single strand PCR reaction by Big Dye Terminator (55.5%). Each sample was amplified in a new 25 µl PCR reaction and sequenced using the same forward primers by Microgene (Korea).

The Statistical Analysis System- SAS (2012) (15) program was used to study the relation between the presence of variations in inflammation bowel and colorectal tumor tissues using Chi-square test. All P-values < 0.01 were suppose statistically significant.

3. Results

3.1 Patients and disease

Thirty six colorectal diseased patients were investigated, including 18 samples with CRC and 18 samples with bowel inflammation. Table 1 showed the clinical character of patients, the mean age of the colorectal cancer patients was 57 years the average age were (46-68) years, 55.5% (10 cases) were females and 44.4% (8 cases) represented males. The severity of the tumor was shown to be moderately differentiated adenocarcinoma 55.5 %(10 cases), well differentiated adenocarcinoma well 33.3% (6 cases) and 11.1% (2 case) represented the poor differentiated adenocarcinoma. The mean age of the inflammation bowel patients was 57 years the average age were (44-65) years, 55.5% (10 cases) were male and 44.4% (8 cases) represented females.

Table 1: Clinical character of patients with colorectal disease

Characterization of tumor patients	*Total no. (%)*
All patients	18 (100)
Mean age	57 years
50<	8 (44.4)
50>	10 (55.6)
	Gender
Male	8 (44.4)
Female	10 (55.6)
	Site of tumor
Colon	8 (44.4)
Rectum	6 (33.3)
Rectosigmoidal	4 (22.2)
	Differentiation
Moderately	10 (55.5)
Well	6 (33.3)
Poorly	2 (11.1)
Characterization of inflammation patients	Total no. (%)
All patients	18 (100)
Mean age	57 years
50<	7 (38.9)
50>	11(61.1)
Gender	
Male	10 (55.6)
Female	8 (44.4)

Detection of mt tRNA variations

Amplified mt tRNA$^{Leu(CUN)}$ gene region using PCR technique and screened for the presence of mutations in 36 colorectal disease patients, the results showed that the product size were about 521 bp Figure (1).

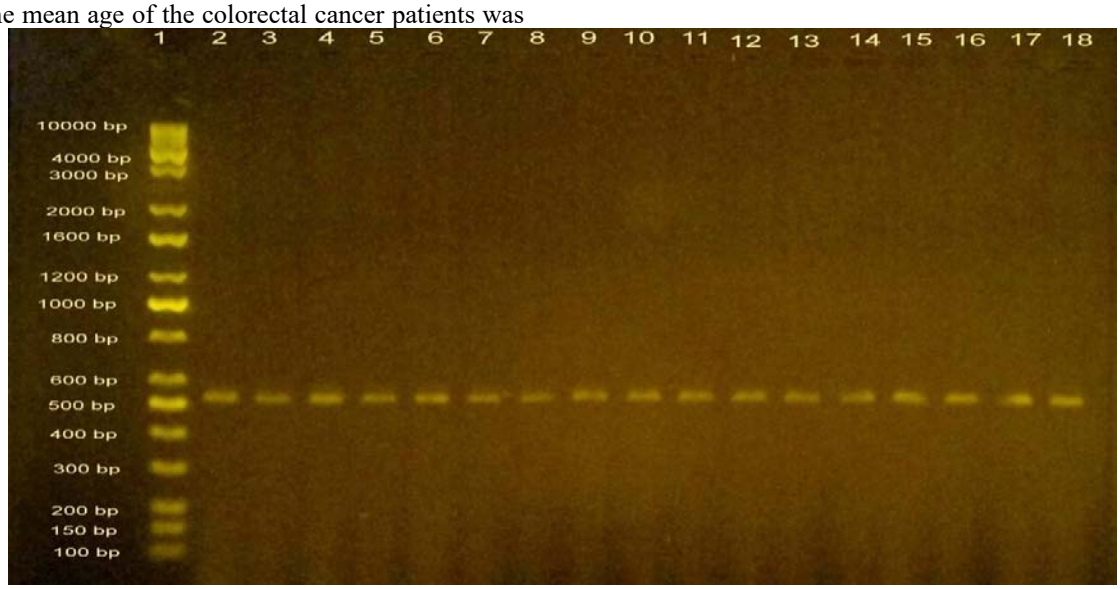

Figure 1: PCR products for mt DNA samples of colorectal disease on 1.2 % agarose gel. Molecular markers, line (1) and mtDNA patients samples, lines (1-18).

To investigate the variation status of mtDNA A12308G tRNA$^{Leu(CUN)}$ gene in colorectal patients, direct sequencing was performed after amplified mtDNA in tRNA$^{Leu(CUN)}$ region in all cases, the sequences analysis results was analyzed by compared to the Human Mitochondrial Reference Sequence NC_012920 published by the National Center for Biotechnology Information (NCBI) data base. The results showed mt tRNA variations in 18 colorectal patients including 10 in CRC patients and 8 in inflammation bowel patients. High rate of mt tRNA$^{Leu(CUN)}$ gene mutations with total of 50 nucleotide variations or differences were presence in both inflammation bowel and CRC.The total frequency of the A12308G transition were found 6 variations out of 18 patients (33.3%), of the 6 nucleotide variations, 3 out of 8 (37.51%) were in inflammation patients and 3 out of 10 (30%) in CRC but these differences were not statistically significant (Table 2),the Figure 2 showed A12308G transition in mt tRNA$^{Leu(CUN)}$. In present results surprising similar distribution for the frequency of the G12372A transition mutations were found in combination with A12308G polymorphism patients. The G12372A variation which was found in NADH dehydrogenase (*ND5* gene) site converts an G to A at nucleotide position 12372, causing the substitution of amino acid position 12 ,Tyr to Tyr which is synonymous change resulted from code changing CTG>CTA (16). The results also indicate to that the A12410C polymorphism was appear in 7out of 18 patients (38.7%), it was presence in 4 out of 8 (50%) in bowel inflammation patients and it was appear in 3 out of 10 (30%) in CRC patients, this variation were coexisted with 7/18(38.7%) frequency of T12414A in same patients distributed as 50% in inflammation bowel patients and 30% in CRC patients, this difference is statistically significant P<0.01 (Tables 2,3).

Table 2: Percentage of mitochondrial (tRNA$^{Leu(CUN)}$) DNA variations

Mutation	Position	Total no. 18 and %		Tumor no. Out of 10 and %		Infl. no. Out of 8 and %		Chi-square
C>A	11920	1	5.55%	1	10%			2.047 NS
C>N	11920	1	5.55%			1	12.5%	3.178 NS
Del A	11924	10	55.5%	4	40%	6	75%	7.449 **
C>T	12043	1	5.55%	1	10%			2.047 NS
C>T	12242	1	5.55%	1	10%			2.047 NS
A>G	12308	6	33.3%	3	30%	3	37.5%	2.612 NS
G>A	12372	6	33.3%	3	30%	3	37.5%	2.612 NS
A>G	12373	1	5.55%			1	12.5%	3.178 NS
Del fragment	12380	1	5.55%	1	10%			2.047 NS
Del fragment	12383	1	5.55%	1	10%			2.047 NS
A>C	12410	7	38.8%	3	30%	4	50%	8.750 **
C>T	12413	1	5.55%	1	10%			2.047 NS
T>A	12414	7	38.8%	3	30%	4	50%	8.750 **
Ins T	12413-12414	4	22.2%	2	20%	2	25%	1.048 NS
Ins C	12413-12414	2	11.11%	1	10%	1	12.5%	0.446 NS
Ins A	12413-12414	1	5.55%	1	10%			2.047 NS
C>A	12417	1	5.55%	1	10%			2.047 NS
** (P<0.01), NS: Non-significant.								

*Infl. no. - inflammation bowel number , Del frag- deletion fragment

High frequency of deletion 11924A base were found in 10 out of 18 samples (55.5%) distributed as 6 out of 8 inflammation bowel patients and 4 out of 10 CRC patients this mutations are statistically significant P<0.01. Total insertions (ins) base in 7 out of 18 colorectal patients (38.8%) between (12413-12414) positions in this work including ins (T, C and A base) but these insertions were non statistical significant (Table 2). Large DNA fragment deletion at (12380...12425) and (12383...12425) positions were presence only in 20% of tumor patients in this work (non statistical significant).

Table 3: Mitochondrial (tRNA$^{Leu(CUN)}$) DNA variations position observed in colorectal disease patients

Tumor no.	Mutations Position								
	C>A	C>T	A>G	G>A	Del _A	A>C	T>A	Del*frag	Ins
1					11924	12410	12414		T/ 12413-12414
2	11920								C/ 12413-12414
3		12242	12308	12372					
4			12308	12372					
5		12043				12410	12414		
6	12417					12410	12414		A/ 12413-12414
7					11924			12380--	
8			12308	12372	11924			12383--	
9		12413				12410	12414		
10					11924				
Infl no.									
1					11924				
2			12373		11924				

3			12308	12372	11924				C/ 12413-12414
4			12308	12372		12410	12414		T/ 12413-12414
5					11924	12410	12414		T/ 12413-12414
6					11924				
7					11924				
8	12417		12308	12372		12410	12414		T/ 12413-12414

* Del frag- deletion fragment

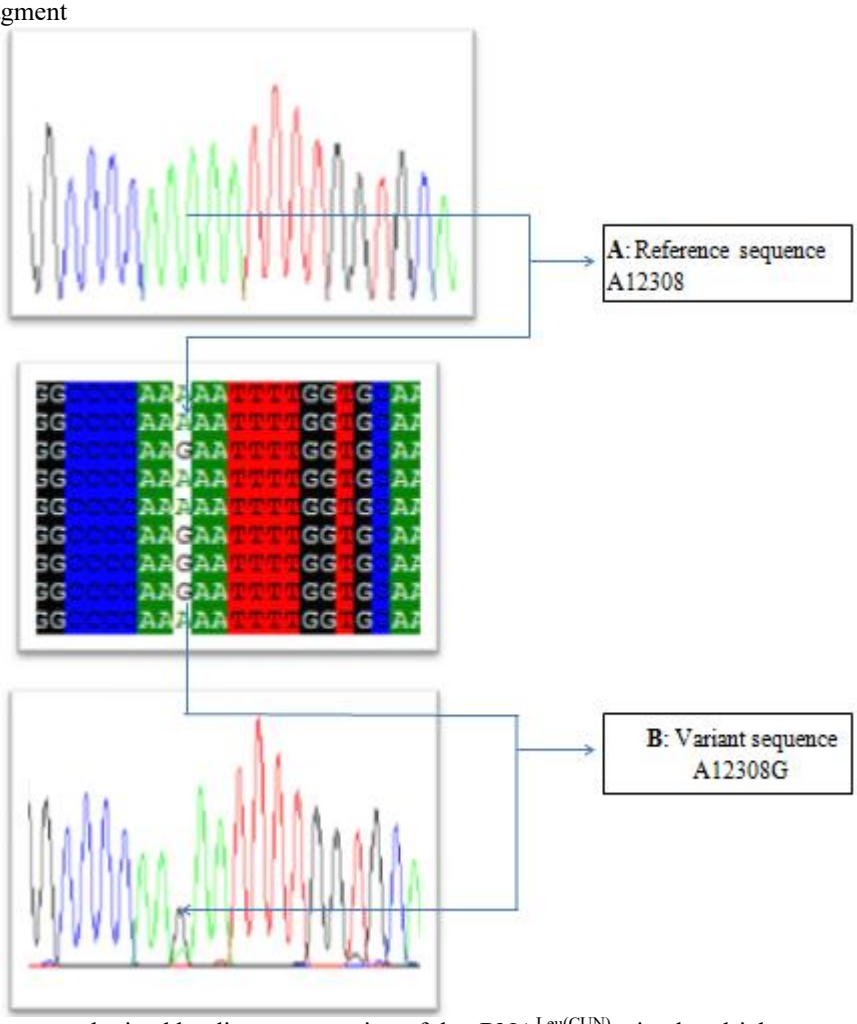

Figure 2: Electropherograms obtained by direct sequencing of the tRNA$^{Leu(CUN)}$ mitochondrial gene region, (A) normal tissue (B) tumor tissue with varient sequence

4. Discussion

Mitochonderial DNA alterations analysis has become a beneficial tool to survey the molecular role of many disease (17, 18).Several articles suggest that mtDNA mutations may cause inherent risk factor for CRC and may serve as a valuable biomarker for prediction of prognosis as well as treatment response and were described to be important markers for CRC outcome evaluation patients (19, 20, 21).

Inflammation associated colorectal carcinogenesis is though results as a step-wise progression from inflamed and hyperplastic, to flat dysplasia epithelia, leading to adenocarcinoma (22). It was found that mitochondrial loss precedes the development of dysplasia, and it could be used to detect and potentially predict cancer, inflammatory bowel disease showed dysplasia arises in precancerous fields of normal tissue which harbors molecular variations and then it progresses from low-grade dysplasia to high-grade dysplasia and cancer (23). This is represent an excellent model to study mitochondrial dysfunction resulted from reactive oxygen species are likely to cause inflammation contribute to mitochondrial damage then cancer progression, the mtDNA variations presence in inflammation bowel patients may be use as genetic tools to detect early tumorgenesis in those patients(24). To the best of our information, this is the first article was conducted to explore the etiology role of the mtDNA A12308G alteration in tRNA$^{Leu(CUN)}$ gene as mtDNA variations pathway in colorectal tumorgenesis on Iraqi patients suffered from bowel inflammation (as known predisposes colorectal cancer) and other group of patients have CRC.

The mtDNA A12308G tRNA$^{Leu(CUN)}$ gene variant in present study may be don't have etiology role in malignant process and these results consistent with other researcher whose suggested that A12308G variant may be a common polymorphism and may not have active functions in patients with thyroid cancer(25, 26).The function of mtDNA A12308G tRNA variations is still controversial, the A12308G position was referred as a main factor that increase risk in several cancers like prostate and kidney cancers (27) and oral

cancer (28). Eslamizadeh *et al*., investigate total mitochondrial tRNAs genes in thirty patients with breast cancer , A12308G a polymorphic alteration was found in 23% breast tumor tissues and 3% in healthy blood controls, they suggested that A12308G alterations may impact on the mitochondrial tRNA structure causing high risk of breast cancer (29). The A12308G variant in tRNA gene have been reported as the most mutation to be associated with mitochondrial diseases , it was reported in patients with congenital cataract by Roshan and colleagues (30) .Others indicate that A12308G polymorphism may have a harmful effect on mtDNA in single mtDNA macrodeletion patients (31). Deschauer *et al.* considered that this mutation may not increase the risk of stroke in patients with the $tRNA^{Leu(UUR)}$ gene alterations (32).

Coincidence of A12308G with G12372A mutations in addition to that in combination of A12410 C with T12414A mutations that appear in this study, can explain by that these two positions, (12308-12372) and (12410-12414) may be having synergistic effect in mtDNA and associated with risk factor in colorectal cancer.

High rate of novel somatic mutations were explored in this study, including a high frequency of deletion 11924A base indicating that 11924-A base is important polymorphism in colorectal disease and can be use as predictive marker that increase risk associated with colorectal tumorgenesis . Figure (3) showed the hot spots of $tRNA^{Leu(CUN)}$ (12308-12425) sequences region in mitochondrial DNA in this work. These data consistent with previous article which indicate to that mtDNA mutations have been reported as several hot spots in tumor tissue but a most target was described in the non-coding D-loop at the D310 sequence (CnTC6) (33), which contains essential transcription and replication elements (34). The majority of mt DNA variations were nucleotide substitutions or single base pair insertions in NADH dehydrogenase subunits, rRNA cytochrome b, and cytochrome oxidase subunits (35, 36), on the other hand more than half of mitochondrial mutations have been located in mt tRNA genes which are hot-spots for mitochondrial pathogenesis, have impact on the secondary and tertiary tRNA structure and may consequently cause transcriptional and translational defects and mitochondrial respiratory chain dysfunction (37).

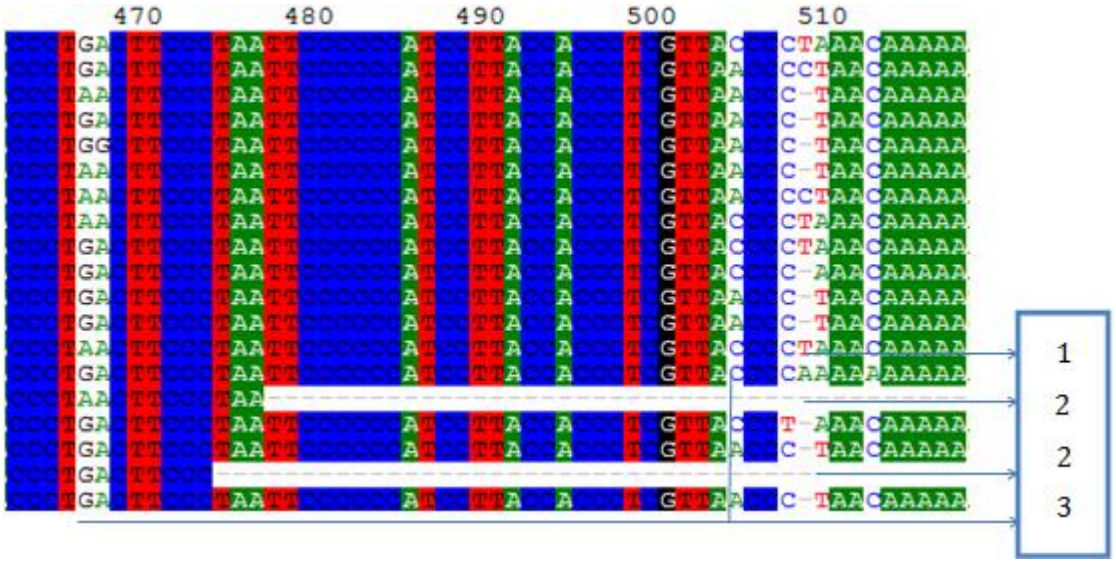

Figure 3: Alignment of sequences for the hot spots region between (12308-12425) sequences in the mtDNA $tRNA^{Leu(CUN)}$ inluding (1) insertion mutations (2) fragment deletion and (3) single base mutations (G>A and A>G) (A>C)

It is well known that mtDNA is more susceptible to alterations , it is error-prone than nuclear DNA because of lacking the protective histones and chromatin structure as well as paucity of introns drive to inefficient mtDNA repair system and is exposed to reactive oxygen species (ROS) produced by oxidative phosphorylation (38, 39) . The metabolic change from oxidative phosphorylation to glycolysis pathway resulted from mtDNA genetic variation like point mutations, deletions and insertions which may result in a compensatory increase in mitochondrial replication and gene expression supporting cancer progression (40).These factors pull together the assemblage of mutations in mtDNA at an about tenfold greatest rate than in nuclear DNA in coding and non-coding regions of mitochondria (41) which may encourage cancer progression (42). Novel germline mitochonderial alterations in the ATPase 8 gene G8573A were seen in tubulovillous adenomas tissues, proposing that some specific mtDNA variants may use as a potential genetic marker for colorectal adenomatous polyps (43, 44). This impact achieved by apoptosis prevention and encourage the generation of cancer-concerning proteins (45).The findings of many mutations in bowel inflammation in this study may interpreted, by since the intestine harbors huge numbers of enterobacteria that constantly induce leucocytes, the constitutive occurrence of inflammation in colorectal mucosa possibly cause the generation of free radicals, thereby inducing the high level of mtDNA variation (46).

Large DNA fragment deletion at (12380...12425) and (12383...12425) positions in 20% of tumor patients were found , consistent with this notion, large deletions of sequence have been found previously in mtDNA from cirrhotic liver surrounding hepatic tumor (47) while Nishikawa *et al.*, attempts to find deletions in mtDNA but

they could not detect such deletions in the colorectal mucosa from patients with ulcerative colitis (48).

In conclusions: mtDNA A12308G $tRNA^{Leu(CUN)}$ gene variants have not impact role in malignant process of colorectal. Coexistence of A12308G with G12372A mutations and combination of A12410 C with T12414A may have synergistic effect in mtDNA function and may increase risk factor in colorectal cancer. These alterations may serve as etiology and supporting factor in colorectal tumorgensis. The most novel mutation were found in this study is deletion 11924 A in mt $tRNA^{Leu(CUN)}$ gene which may be use as genetic biomarker in colorectal cancer. Data from similar work target other mt tRNA genese in large number of Iraqi patients are required to clarify the mechanism of carcinogenosis as mt DNA variations pathway.

References

[1] Cui H, Huang P, Wang Z, Zhang Y, Zhang Z, Xu W, Wang X, Han Y, Guo X. (2013) Association of decreased mitochondrial DNA content with the progression of colorectal cancer. BMC Cancer, 13:110.
[2] Marcelino LA, Thilly WG. (1999) Mitochondrial mutagenesis in human cells and tissues. N`mbv cMutat Res 434:177–203.
[3] Guleng G, Løvig T, Meling GI, Andersen SN, Rognum TO.(2005) Mitochondrial microsatellite instability in colorectal carcinomas–frequency and association with nuclear microsatellite instability. Cancer Lett, 219:97–103.
[4] Gredilla R, Bohr VA, Stevnsner T. (2010) Mitochondrial DNA repair and association with aging—an update. Exp Gerontol, 45:478–488.
[5] MiwataT, Hiyama T, Quach DT, Le HM, Hua HNT, Oka S, Tanaka S, Arihiro K and Miwata KC et al.(2014) Differences in K-ras and mitochondrial DNA mutations and microsatellite instability between colorectal cancers of Vietnamese and Japanese patients .BMC Gastroenterology. 14:203.
[6] Holt IJ, Harding AE, Morgan Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies.(1988) Nature, 331:717–9.
[7] Kudryavtseva AV, Lipatova AV, Zaretsky AR, Moskalev AA, Fedorova MS, Rasskazova AS, Shibukhova GA, Snezhkina AV, Kaprin AD, Alekseev BY, Dmitriev AA, Krasnov GS.(2016) Important molecular genetic markers of colorectal cancer. Oncotarget, 7:53959-53983.
[8] Carethers JM and Jung BH.(2015) Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer. Gastroenterology, 149:1177-1190.
[9] Shin US, Cho SS, Moon SM, Park SH, Jee SH, Jung EJ, et al.(2014). Is microsatellite instability really a good prognostic factor of colorectal cancer? Ann Coloproctol. 30:28-34.
[10] Vogelaar F, Van Erning F, Reimers M, Van Der Linden J, Pruijt J, Van Den Brule A, et al.(2015).The prognostic value of Microsatellite Instability, KRAS, BRAF and PIK3CA mutations in stage II colon cancer patients. Mol Med. 17:1-26.
[11] Iachetta F, Domati F, Reggiani-Bonetti L, Barresi V, Magnani G, Marcheselli L. et al.(2016) Prognostic relevance of microsatellite instability in pT3N0M0 colon cancer: a population-based study. Intern Emerg Med. 11:41-46.
[12] Li X, YaoX, Wang Y, Hu F, Wang F, Jiang L, Liu Y, Wang D, Sun G, Zhao Y. (2013) MLH1 Promoter Methylation Frequency in Colorectal Cancer Patients and Related Clinicopathological and Molecular Features .PLOS ONE . 8: e59064.
[13] Gredilla R, Bohr VA, Stevnsner T. (2015): Mitochondrial DNA repair and association with aging—an update. Exp Gerontol. 45:478–488.
[14] Mohammed FM, khorasany AR, Mosaieby E and Houshmand M.(2015). Mitochondrial A12308G alteration in tRNALeu(CUN) in colorectal cancer samples. Diagnostic Pathology, 10:115.
[15] SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
[16] Tanwar M, Dada T, Sihota R. and Dada R. (2010). Mitochondrial DNA analysis in primary congenital glaucoma. Molecular Vision . 16:518-533
[17] Chatterjee A, Mambo E and Sidransky D. (2006) Review: Mitochondrial DNA mutations in human cancer. Oncogene, 25: 4663–4674.
[18] Mohideen AM, Dicks E, Parfrey P, Green R, Savas S.(2015) Mitochondrial DNA polymorphisms, its copy number change and outcome in colorectal cancer. BMC Res Notes 8:272.
[19] Govatati S, Saradamma B, Malempati S, Dasi D, Thupurani MK, Nagesh N, Shivaji S, Bhanoori M, Tamanam RR, Nallanchakravarthula V, Pasupuleti SR. (2016) Association of mitochondrial displacement loop polymorphisms with risk of colorectal cancer in south Indian population. Mitochondrial DNA A DNA Mapp Seq Anal. 17:1-6.
[20] Qu F, Chen Y, Wang X, He X, Ren T, Huang Q, Zhang J, Liu X, Guo X, Gu J, Xing J.(2015). Leukocyte mitochondrial DNA content: a novel biomarker associated with prognosis and therapeutic outcome in colorectal cancer. Carcinogenesis. 36:543-52.
[21] Wang C, Zhao S, Du Y, Guo Z. (2015) Single nucleotidepolymorphisms in the D-loop region of mitochondrial DNA is associated with colorectal cancer outcome. Mitochondrial DNA. 6:1-3.
[22] Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC. (1983) Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum. Pathol. 14: 931–966.
[23] Ussakli CH, Ebaee A, Binkley J, Brentnall TA, Emond MJ, Rabinovitch PS, Risques RA. (2013) Mitochondria and Tumor Progression in Ulcerative Colitis. J Natl Cancer Inst. 105:1239-48
[24] Shimomura T, Hiyama T, Oka S, Tanaka S, Yoshihara M, Shimamoto F, Chayama K. (2011) Frequent somatic mutations of mitochondrial DNA in traditional serrated adenomas but not in sessile serrated adenomas of the colorectum. J Gastroenterol Hepatol. 26:1565-9.
[25] Houshmand M, Larsson NG, Holme E, Oldfors A, Tulinius MH, Andersen O.(1994) Automatic sequencing

of mitochondrial tRNA genes in patients with mitochondrial encephalomyopathy. BBA-Mol Basis Dis. 1226:49–55.

[26] Lv F, Qian G, You W, Lin H, Wang XF, Qiu GS, Jiang YS, Pang LX, Kang YM, Jia BF, Xu JZ, Yu Y .(2015) Variant in mitochonderiah tRNAgene may not be associated with thyroid carcinoma. Blakan J Med. Gen. 18: l 59-164.

[27] Booker LM, Habermacher GM, Jessie BC, Sun QC, Baumann AK, Amin M, et al. (2006) North American white mitochondrial haplogroups in prostate and renal cancer. J Urology. 175:468–73.

[28] Datta S, Majumder M, Biswas NK, Sikdar N, Roy B (2007). Increased risk of oral cancer in relation to common Indian mitochondrial polymorphisms and Autosomal GSTP1 locus. Cancer. 110:1991–9.

[29] Eslamizadeh S, Ghaffarpour M, Arabzadeh M, Houshmand M.(2014) Mitochondrial 22 tRNAs alterations in Iranian breast cancer patients. [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research;. Philadelphia (PA): AACR; Cancer Res. 74:(19 Suppl)

[30] Roshan M., Shama Prasada Kabekkodu S P. Vijaya P H , Manjunath K, Graw J , Gopinath PM. , Satyamoorthy K. (2012) Analysis of mitochondrial DNA variations in Indian patients with congenital cataract. Molecular Vision , 18:181-193

[31] Crimi M, Del Bo R, Sara Galbiati1, Monica Sciacco1, Andreina Bordoni1, Nereo Bresolin1,2 and Giacomo Pietro Comi .(2003) Mitochondrial A12308G polymorphism affect clinical features in patients with single mtDNA macrodeletion. European Journal of Human Genetics 11: 896–898 .

[32] Deschauer M, Chinnery P F , Schaefer A M, Turnbull D M, Taylor R W, Deschauer M, Zierz S, Shanske S, DiMauro S, Majamaa K, Wilichowski E and Thorburn DR.(2004) No association of the mitochondrial DNA A12308G polymorphism with increased risk of stroke in patients with the A3243G mutation . J Neurol Neurosurg Psychiatry ,75:1200–1207.

[33] Sanchez-Cespedes M, Parrella P, Nomoto S, Cohen D, Xiao Y, Esteller M, Jeronimo C, Jordan RC, Nicol T. and Koch WM. (2001) Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. Cancer Res. 61:7015– 7019.

[34] Larman TC, DePalma SR, Hadjipanayis AG, Cancer Genome Atlas Research N, Protopopov A, Zhang J, Gabriel SB, Chin L, Seidman CE, Kucherlapati R . and Seidman JG. (2012) Spectrum of somatic mitochondrial mutations in five cancers. Proc Natl Acad Sci U S A 109: 14087–14091

[35] Marcelino LA and Thilly WG. (1999) Mitochondrial mutagenesis in human cells and tissues. Mutat Res. 434:177–203

[36] Zifa E, Theotokis P, Kaminari A, Maridaki H, Leze H, Petsiava E, Mamuris Z, Stathopoulos C. (2008) A novel G3337A mitochondrial ND1 mutation related to cardiomyopathy co-segregates with $tRNA^{Leu(CUN)}$ A12308G and tRNAThr C15946T mutationsMitochondrion. 8:229-36.

[37] Sternberg D, Danan C, Lombès A, Laforêt P, Girodon E, Goossens M, *et al.* 1998. Exhaustive scanning approach to screen all the mitochondrial tRNA genes for mutations and its application to the investigation of 35 independent patients with mitochondrial disorders. Hum Mol Genet. 7: 33-42.

[38] Park SY, ShinMG, Kim HR, Oh JY, KimSH, Shin JH, Cho YB, Suh SP, Ryang DW. (2009) Alteration of mitochondrial DNA sequence and copy number in nasal polyp tissue. Mitochondrion, 9:318–325

[39] Singh KK, Kulawiec M.(2009). Mitochondrial DNA polymorphism and risk of cancer. Methods Mol Biol . 471:291–303.

[40] Wallace DC. (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet. 39:359–407.

[41] Modica-Napolitano JS, Kulawiec M, Singh KK.(2007) Mitochondria and human cancer. Curr Mol Med . 7:121–131.

[42] Lee HC, Chang CM, Chi CW. (2010) Somatic mutations of mitochondrial DNA in aging and cancer progression. Ageing Res Rev. 9(Suppl1):S47–S58

[43] Lee JH, Hwang I, Kang YN, ChoiI J, Kim DK.(2015) Genetic Characteristics of Mitochondrial DNA Was Associated with Colorectal Carcinogenesis and its prognosis .PLOS ONE, 10:e0118612.

[44] Gregory AJR. , Mehrabi S, Vatcharapijarn Y , Iyamu OI , Akwe JA, Grizzle W E, Yao X, and Aikhionbare FO.(2013) Frequencies of mtDNA mutations in primary tissue of colorectal adenopolyps. Front Biosci (Elite Ed). 1:5:809-13.

[45] Kose K, Hiyama T, Tanaka S, Yoshihara M, Yasui W, Chayama K. J (2005) Somatic mutations of mitochondrial DNA in digestive tract cancers. Gastroenterol Hepatol. 20:1679-84.

[46] Nishikawa M, Oshitani N, Matsumoto T, Nishigami T, Arakawa T and Inoue M.(2005) Accumulation of mitochondrial DNA mutation with colorectal carcinogenesis in ulcerative colitis British Journal of Cancer,93: 331 – 337.

[47] Yamamoto H, Tanaka M, Katayama M, Obayashi T, Nimura Y, Ozawa T. (1992) Significant existence of deleted mitochondrial DNA in cirrhotic liver surrounding hepatic tumor. Biochem Biophys Res Commun 182: 913–920.

[48] Nishikawa M, Oshitani N, Matsumoto T, Nishigami T, Arakawa T and Inoue M. (2005) Accumulation of mitochondrial DNA mutation with colorectal carcinogenesis in ulcerative colitis British Journal of Cancer 93: 331 – 337.