UNIVERSITY OF GAZIANTEP

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

LOSS OF HETEROZYGOSITY ON 21q11-22 REGION IN COLORECTAL CANCER

M.Sc. THESIS

IN

BIOLOGY SCIENCE

BY

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June 2014

Loss of Heterozygosit on 21q11-22 Region in Colorectal Cancer

M.Sc. Thesis

In

Biology Science

Supervisor

Prof. Dr. Mehmet OZASLAN

BY

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June 2014

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I have fully cited and referenced all material and result that are not original to this work.

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REPUBLIC OF TURKEY

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BIOLOGY DEPARTMENT

Name of the thesis: Loss of Heterozygosity on 21q11-22 Region in Colorectal Cancer

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Exam date: 02.06.2014

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ABSTRACT

LOSS OF HETEROZYGOSITY ON 21q11-22 REGION IN COLORECTAL CANCER

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M.Sc. in Biology Department

Supervisor: Prof. Dr. Mehmet OZASLAN

June 2014, 55 Pages

Colorectal cancer (CRC) is the most common cancers in western populations. In Iraq, a developing Asian country in the Eastern Mediterranean region, these rates reach four times less than in developed countries. The pathogenesis of CRC is very complex and diverse and it's also influenced by multiple processes. The detection of loss of heterozygosity (LOH) of tumor suppressor genes and deletion chromosome associated with colorectal cancer, so the microsatellite DNA loci are useful markers for which aims in our study. The present study examined 5 polymorphic microsatellite markers in along chromosome 21q11-22 region matched in 42 patients of CRC (tumor and normal) tissues identified frequency loss of heterozygosity of each loci of markers, Comparisons between LOH frequency and some of pathological etiology of the sampled tissues were performed by fisher's exact test, $P<0.05$ was considered as statistically significant. We get 29 (%57, 1) patients were detected LOH from sum of 42 cases at least in one locus. The highest frequency of LOH was found on D21S1839 (%19) loci. The lowest frequency of LOH was on D21S270 (%7, 1) loci. The occurrence of LOH in region 21q11-22 was extremely significant with gender (P=0.0001). It is concluded that LOH in region 21q11-22 is found to be associated with CRC in females.

Keywords: Colorectal cancer, loss of heterozygosity, chromosome 21

ÖZET

KOLOREKTAL KANSERLİ HASTALARDA 21q11-22 BÖLGESİ LOH ANALİZİ

ALBARAWI, Dilan

Yüksek Lisans Tezi, Biyoloji Bölümü

Tez Yöneticisi : Prof. Dr. Mehmet ÖZASLAN

Haziran 2014, 55 sayfa

Kolorektal kanser batı ülkelerinde en yaygın görülen kanser türüdür. Doğu AkdenizBölgesi' nde gelişen bir Asya ülkesi olan Irak' ta bu oran gelişmiş ülkelerden dört katdaha azdır. Kolorektal kanserin patogenezi oldukça kompleks, farklı ve çeşitlifaktörlerle etkilenir. Kolorektal kanserle ilişkili tümör baskılayıcı genlerde meydanagelen heterozigotluk kaybının (LOH) ve kromozomal delesyonun tespiti vemikrosatellit DNA lokusu, hedeflediğimiz çalışma için yararlı markırlardır. Hedeflenen çalışmada kromozom 21q11-22 bölgesinde yer alan 5 polimorfik markırkullanılarak 42 kolorektal kanserli hastadan alınan tümörlü ve normal dokuda herlokus için heterozigotluk kaybı analizi yapılmıştır. Çalışma sonucunda toplam 42hastanın 29' unda (%57,1) en az bir lokusta LOH saptanmıştır. En yüksek LOH sıklığıD21S1839 (%19) lokusunda tespit edilmiştir. En düşük LOH sıklığı ise D21S270 (%7,1) lokusunda saptanmıştır. 21q11-22 bölgesinde LOH'un ortaya çıkış oranı cinsiyet ile önemli ölçüde (P=0.0001) değişmektedir. Kadınlarda 21q11-22 bölgesindeki LOH CRC ile ilişkili olarak düşünülmektedir.

Anahtar Kelimeler: Kolorektal kanser, heterozigotluk kaybı, 21. kromozom

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my Supervisor Prof. Dr. Mehmet ÖZASLAN for the continuous support of my M.Sc. study, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

It is an honor for me to show my thanks and appreciation to Gaziantep university for giving me the opportunity to study M.Sc. , and I am grateful to all teachers, colleagues and the staff in a department of molecular biology, for help me along time to complement my study.

I am deeply grateful to Assist. Prof. Dr. I. Didem KARAGOZ, she was always willing to help and give her best suggestions.

I would especially like to thank my bosses in Duhok University/ Center of Scientific Research: Prof. Dr. Jaladet MS. Jubrael, Prof. Dr. Farida Fariq Nerweyi and Asa'ad Abdlwahd Alasady whom supported me, also I thankful for department of Histopathology - Duhok Central lab were provided me samples for this study.

I express my thanks to my dear friend Ceyda UYAR, whose was helped me at a begin in laboratory.

I wish to thank my best friends Nawal AL-AAZI and Jagar Mustafa Ismail for help me get through the difficult times, for all the support, entertainment, and caring they provided.

I would like to thank my family brothers, sisters and specially my parents, for giving birth to me at the first place and supporting me spiritually throughout my life.

"Last but not least, thanks be to God for my life through all tests in the past two years"

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LIST OF SYMBOLS/ABBREVIATIONS

21q	Chromosome 21
ACF	Aberrant crypt foci
ACO2	Aconitase 2
AFAP	Attenuated familial adenomatous polyposis
AJCC	American Joint Committee on Cancer
AML	Acute myeloid leukemia
APC	Adenomatous polyposis coli gene
bp	Base pair
CDKN1A	**C**yclin-dependent kinase inhibitor 1A
CDKs	Cyclin-dependent kinases
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CpG	Cytosine nucleotide occurs next to a guanine nucleotide
CRC	Colerectala cancer
DCC	Deleted in colorectal cancer gene
DNA	Deoxyribo nucleic acid
EDTA	Ethylenediaminetetraacetic acid
elF	Eukaryotic initiation factors
FAP	Familial adenomatouse polyposis
G1	Gastrointestinal
GIT	Gastrointestinal tract
HNPCC	Hereditary nonpolyposis colorectal cancer
K-ras	Kirsten rat sarcoma viral oncogene homolog
LOH	Loss of heterozygosity

MAP	MUTYH associated polyposis
MAPK	Mitogen-activated protein kinase
MDM2	Mouse double minute 2 homolog
MLH1	MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)
MMR	Mismatch repair
MSH2	DNA mismatch repair protein
MSH6	mutS homolog 6
MSI	Microsatellite instability
MSS	Microsatellite Stable
Mtor	Mammalian target of rapamycin
NCI	National cancer institue
NI	Non informative
OSCC	Oral squamous cell carcinoma
PAGE	Polyacrilamid gel electrophoresis
PCR	Polymerase chain reaction
PIK13	Phosphatidylinositol 3-kinase
PIK13CA	Phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic
PMS2	Mismatch repair endonuclease
RLOH	Retintion loss of heterozygosity
RNA	Ribonucleotide acid
RUNX1	Runt-related transcription factor 1
RUNX2	Runt-related transcription factor 2
RUNX3	Runt-related transcription factor 3
SEER	Surveillance, epidemiology, and end Results
SMAD4	Mothers against decapentaplegic , drosophila, homolog of, 4
SSRs	Simple Sequence Repeats
STRs	Short tandem repeats
TCF	Transcription factor
TEMTED	Tetramethylethylenediamine

TFF3	Trefoil factor 3
TFFI	Trefoil factor 1
TGF-b	Transforming growth factor beta
TNM	Tumor lymph nodes metastasis
TP53	Protein p53
TSGs	Tumor suppressor genes
UC	Ulcerative colitis
UICC	International Union Against Cancer
UV	Ultraviolet

CHAPTER 1
INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the Western countries, even the age standardized rates are the top range in the world, adding has rates much lower in developing countries in Asia & Africa. In Iraq, a developing Asian country in the eastern mediterranean region, these rates reach four times less than those in North America and most European countries (Al-allawi et al., 2012). CRC is the third leading cause of cancer in males and the second among in females. In 2008 Epidemiologists have been reported that the annual worldwide incidence of colorectal cancer was 1.2 million, almost evenly of both genders (females, males). Generally this type of cancer starts in the cells of glandular structures in the inner layer of the colon due to uncontrolled division, which are called polyps, these polyps evolution into adenomatous tissue over time can lead to adenocarcinoma96% of all colorectal cancers were progression from adenomatous tissues and firstly spreads into wall of the colon then by lymphatic system into other organs (National Cancer Institute, 2011). In the past decade, it has been unprecedented progress in reducing colorectal cancer incidence and death rates in most American population groups, this progress has came about significantly through the prevention and early detection of colorectal cancer through screening. (Petrova, at el., 2008) Currently, only about half of people aged 50 or older for whom had taken detection and screening recommended, reports have been received colorectal cancer testing consistent with current guidelines, as well to following recommended screening guidelines, people can decreasing risk of developing or death colorectal cancer by organize life style like maintaining a healthy body weight, regular physical activity, limiting intake of red and processed meats to replace it dietary fiber and try keep away from tobacco. **(**Colorectal Cancer Facts & Figures 2011-2013)**.**

Chromosome 21 is the smallest human autism, spanning about 48 million base pairs (the known essential building blocks of deoxyribo nucleic acid DNA) and representing 1.5 to 2 percent of the total DNA in cells. In 2000, researchers working on the human genome project announced that they had identified the sequence of base pairs which consists this chromosome. Chromosome 21 was the second human chromosome to be fully sequenced. Assign genes on each chromosome is an active area of genetic researches. Because researchers use different approaches to looking forward the number of genes on each chromosome, the estimated number of genes varies. Chromosome 21 probably contains 200 to 300 genes and 20,000 to 25,000 total genes in the human genome (http: //ghr. nlm. NIH. Gov/chromosome/21). An extra copy of chromosome 21 causes Down syndrome, which affects up to 1 in 700 live births. Many anonymous loci for monogenic disorders and standby for common complex disorders have also been mapped to this chromosome and loss of heterozygosity has been observed in regions associated with solid tumors and cancers (Hattori et al., 2000).

Loss of heterozygosity (LOH) is defined as the loss normal function of one allele at a locus, often by deletion in which the other allele had been already inactivated, a gremline mutation is passed on from one of the parents and the inactivation or breakdown by LOH is characteristic of the loss of tumors suppressor genes, (Machado et al., 2010**).** The loss of heterozygosity (LOH) in tumor suppressor genes is believed one of the key step in general carcinogenesis and in colorectal cancer particularly, loss of one allele at a specific locus is caused by a deletion, mutation or loss of a chromosome in chromosome pair, when this occurs in a tumor suppressor gene locus where one of the alleles is already abnormal, it can result in neoplastic transformation (Zhou et al., 2002).

Microsatellite markers, also called simple sequence repeats (SSRs) or short tandem repeats (STRs) are polymorphic DNA loci consisting of a repeated nucleotide sequence, the repeat sequence can be from 2- 7 base pairs long, the number of repeat unit variation in a population thereby are creating multiple alleles for a microsatellite locus (the GeneMapper, 2009). SSRs are a highly variable number, hundreds thousands of such pieces of the sequences were found over the human genome, SSRs are effective and successful markers to identify alleles at linked loci of genes and then can be used in linkage studies, segregation analysis and in investigating the somatic loss of heterozygosity or allele imbalance in tumors **(**Applications of

molecular genetics in personalized medicine) (Kluwe, 2013). An LOH microsatellite analysis is the screening of tumor samples for LOH using microsatellite markers because LOH can be caused by deletion of genomic DNA regions containing the wild-type copy of a tumor suppressor gene (the GeneMapper, 2009).

1.1. What is Cancer

Tumorigenesis in humans is a multistep process and those successive steps reflect genetic changes which lead the progressive transformation of normal human cells into highly malignant derivatives, the type of genes that are target for causative mutations of cancer have abnormal functions and which grant the cell with the mutations to acquire oncogenic capabilities (Hanahan and Weinberg, 2000). Cancer is the outcome of a series of molecular events radical which in turn leads to change the natural characteristics of the cells and destroy them, through the control of it to the natural cell cycle, see figure 1.1 (Schneider, 2001). The leading causes of cancer may be the influence of external, environmental factors such as (exposure to radiation, infectious organisms, smoking, and chemical substances) or internal factors and which also called a genetic factors such as (immune conditions, mutations that occur from metabolism, inherited mutations and hormones), the appearance of these causal factors and overlap could lead to initiate or promote the development of cancer (Cancer Facts & Figures, 2013). Hanahan and Weinberg in 2000, they suggested that several essential changes in cell physiology during cell cycle are responsible for development tumor cells, thus it lose to control cell cycle, differentiation, and programmed cell death, even they lead induce the formation of blood vessels and eventually invade to surrounding tissues and metastasize to other tissue of organs (Zhang and Simon, 2005).

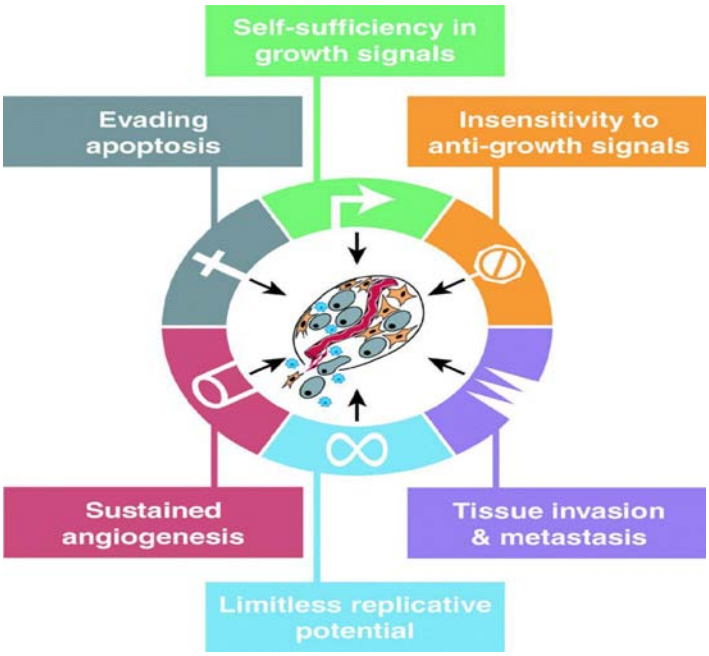


Figure 1.1. Acquired Capabilities of Cancer (Hanahan, 2000).

Unfortunately, most of cancers sporadically which appear by chance in a single cell offering it a growth advantage. (Schneider, 2001). At beginning of the seventies, scientists discovered two of the particularly important families of genes linked to a cancer, they are oncogene genes and tumor suppressor genes (Zhang and Simon, 2005).

a) Oncogene genes:

Oncogenes genes lead the cells to grow out of control and two caused cancer cells, whether the cancer is sporadic or a hereditary could metastasize into normal tissues and characterized by the aggregation of genetic alterations in the target tissue (Zhang and Simon, 2005). They are formed by changes or mutations of some normal or specific genes of the cell called proto-oncogenes. Proto-oncogenes are the genes that normally control how often a cell divides and the degree to which it differentiates (or specializes in a specific function in the body), see figure 1.2, (American cancer society, 2013).

b)Tumor suppressor genes (TSGs):

Tumor suppressor genes can be defined as genes which encode or intensive proteins that inhibit the formation of tumors. Their normal action are to discourage cell growth, or act as the brakes for the cell cycle, mutations in tumor suppressor genes contributes to the development of cancer by inhibition that retardant function, mutations of this kind are termed loss-of-function mutations, as long as the cell

contains one functional copy of a given tumor suppressor gene (expressing enough protein to control cell growth) that gene can inhibit the formation of tumors, see figure 1.2, (American cancer society, 2013).

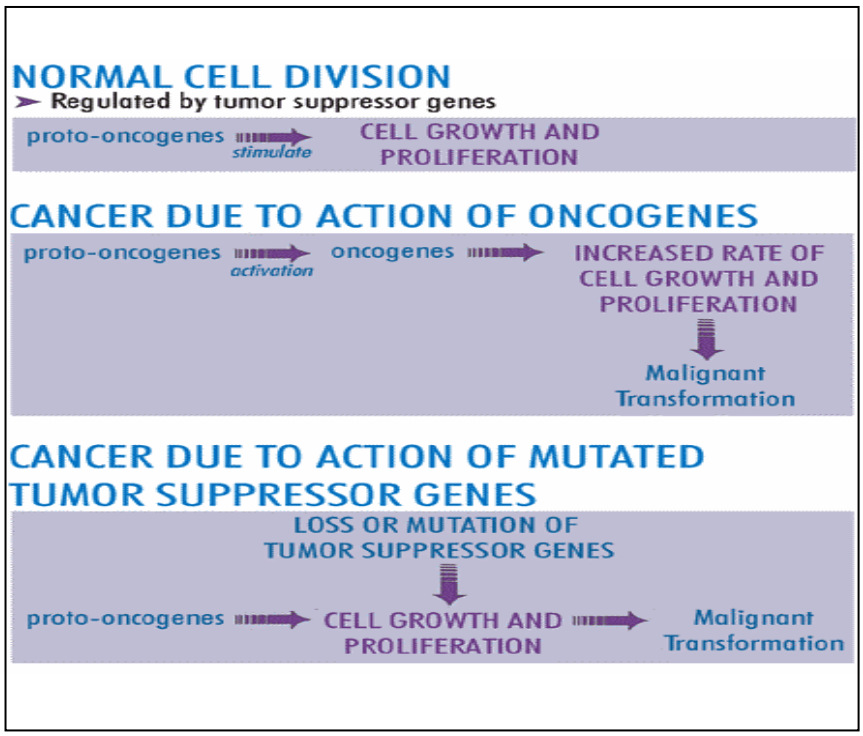


Figure 1.2. The affected proto-oncogenes, oncogenes and tumor suppressor genes on cell division (http://biology.kenyon.edu/)

Accumulated evidence result to that tumors of different types, it can develop into any tissue of the body and named according to the types, cancer can be benign topical noninvasive or malignant invasive metastatic, the metastases produce by malignant tumors are responsible for nearly all deaths resulting from cancer, cancers are classified into four key groups according to their origin (epithelial, mesenchymal, hematopoietic, and neuroectodermal), indeed all cell types in the body can give rise to cancer but the most common human cancers are of epithelial origin the carcinomas, most carcinomas are divided into two categories squamous cell carcinomas arise from epithelia that form protective cell layers and adenocarcinomas arise from secretary epithelia, non-epithelial malignant tumors involve sarcomas which arise from mesenchymal cells, hematopoietic cancers which arise from cells of the circulatory or from the immune systems and neuroectodermal tumors, which arise from components of the nervous system (Wilbure, 2003).

1.2. Colorectal Cancer (CRC)

Colorectal cancer is also called bowel cancer, CRC are colon and also rectum cancer, because research analysis by scientists have the same genetics basically, usually the malignant tumor of colorectal cancer origin from a noncancerous polyp (glandular cells that line the inside of the colon**) (** Armaghany et al, 2012)**.** polyp is a rise of tissue that develops on the lining of the colon or rectum that can convert to cancerous, called adenomatous polyps or adenomas that majority of cases of colorectal cancer caused by adenocarcinomas (Colorectal Cancer Facts & Figures, 2013).

1.3. Biology of the large Intestine

1.3.1. Anatomy of Colon

Common name is large intestine, it is an organ of the digestive system and last part of it, is about (1.5 m) in length and (6.3 cm) in diameter approximately, the large intestine starts at caecum, on the posterior medial wall of which is the appendix, the colon is made up of ascending, transverse, descending and sigmoid part, which join the rectum at the recto sigmoid junction (Armaghany et al, 2012). Structure of layers intestinal wall, begin from within it consist mucosa, submucosa, muscle coat, and serosa or peritoneum, the mucosa is lined with columnar epithelial cells with crypts without villi, so generally it is flat. The mucosa contains full of goblet cells, see figure 1.3, (Feldman, et al., 2010). The main function of the large intestine is absorption of minerals (sodium ions, chloride ions) and water, with removal of undigested food and waste out the body (Carlsson, 2013). Microbial fermentation the large intestine abounding microbial life, those microbes produce enzymes able to decomposition foodstuffs, especially carbohydrates (Armaghany et al, 2012).

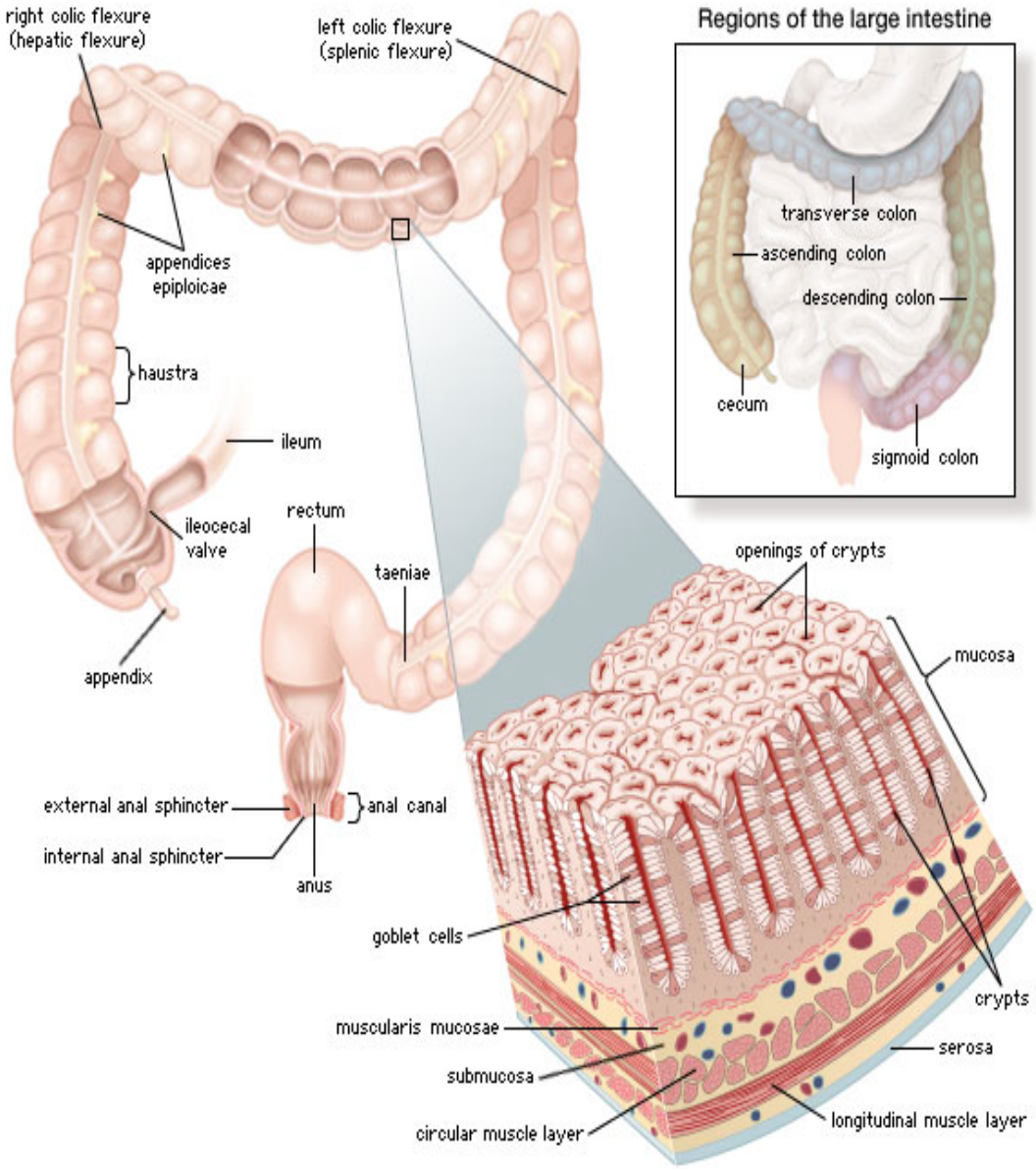


Figure1.3. Anatomy of large intestine (Encyclopedia Britannica Inc., 2003)

1.3.2. Epidemiology of Colorectal Cancer

Colorectal cancer is the third most common cancer diagnosed in men and women and the second leading cause of cancer morbidity and mortality worldwide. In 2011 cases record (141,210) new Patients and 49,380 deaths expected. Almost 72% of cases arise from colon and nearly 28% of rectum in the United States (Colorectal Cancer Facts & Figures, 2013). An analysis of data from the population-based mentions of the national cancer institute (NCI) Surveillance, epidemiology and end results of Surveillance, epidemiology, and end Results (SEER) program achieved an adjusted five year conditional probability of survival and stay alive, they had seen

significantly improve cases with advanced stages of development of the disease, the cases had been almost 80% survival, but cases stage IV disease reached in 48% for adjusted five years conditional probabilities of survival (Luz 2008). Tumors can arise within the walls of the colon tissue, which are called polyps, almost half of the population will develop to benign polyp during the life, down polyps may develop into adenomatous polyps over time and in fact majority about 80% of colorectal cancer developed from an adenomatous polyp (Cidon, 2011). A preneoplastic polyp progressively builds up genetic changes that lead to uncontrolled proliferation and cell survival, followed by invasive and metastatic properties typical of carcinoma (Petrova, 2008). Colorectal cancer so can be sporadic related to environmental factors and lifestyle causes, or inherited relating to inherited predisposition or familial risk (Hankey and Groden, 2013).

1.4. Etiology of Colorectal Cancer

CRC is known to be one more detectable and treatable of other kinds of cancers, and they put a focus on risk factors, the level of awareness is therefore critical and very helpful in decreasing incidence and mortality rates (Murali, 2012). Generally two factors have a role to causes of CRC are both environmental factors and inherited, the mode of presentation of CRC follows one of three patterns that are expression these differing risk factors: sporadic, inherited, and familial (Cheah, 2008).

1.4.1. Sporadic Diseases

Sporadic CRC does not have any link with inherited or family history of the CRC, accounts up to 70% of percentage of all CRCs. It is most common over the age of 50 years CRC has more chance to appearance with increasing age (Murali, 2012). Moreover, incidence rates are 14 times greater for patients over the age of 50 years than those younger (Murali, 2012). Dietary factors and lifestyle high-calorie diet, taking a multitude of animal products, especially meat and saturated fats with low intake of fruits, fibers and vegetable, there are different studies about the relationship between tobacco use and polyp formation there was a weak relationship between dosing and carcinogenesis mechanism (Bruckner et al., 2000). Alcohol consumption of over two drinks per day lead to deficient in methionine and folate, even alcohol users tend to have low levels of folic acid in the body, abnormalities in the methylation of DNA may be the responsible for carcinogenic, diabetes mellitus and

insulin resistance arise evidence have more probability that diabetes mellitus is associated with an increasing risk of CRC, recently, many searches focus has turned from single risk factor analyses towards gene-environment interactions in cancer development (Elshimali et al, 2013). Gene-environment interaction can be defined as a different effect of an environmental exposure on risk of disease in persons with different genotypes, or a different effect of a genotype on risk nine of disease in people with different environmental exposure. Interaction applies when one stratum (whose carrier of the high risk allele) responds differently to an exposure whose person a dietary component than another stratum (person carrier of low risk allele) (Luz, 2008).

1.4.2. Inherited Diseases

Approximately 10% percent of cases have a true inherited predisposition to CRC, and these patients are depending whether or not colonic polyps which are a major disease manifestation, familial adenomatous polyposis (FAP) and its variants (Gardner's syndrome, Turcot's syndrome and attenuated adenomatous polyposis coli) MUTYH associated polyposis (MAP) and the hamartomatous polyposis syndromes (eg, Peutz-Jeghers, juvenile polyposis) while others that are without polyposis are referred to as hereditary (HNPCC, Lynch syndrome) Lynch syndrome as known hereditary nonpolyposis colorectal cancer (HNPCC) is one the diseases fixed in the autosomal dominant with a high lifetime risk for enhancing into CRC, mutations in this syndrome located in DNA mismatch repair (MMR) genes in 50% of all HNPCC families, neither MMR mutations nor microsatellite instability is clearly in which case it is not called lynch syndrome about 2 to 3% of all colorectal cancers are HNPCC usually patients are between (40-50 year) (Koskensalo, 2013).

1.4.3. Familial CRC

The third pattern and least understanding is commonly known as "familial" CRC which accounts for up to 25% percent of patients, affected cases have a family history of CRC, but familial" CRC is not consistent with one of the inherited syndromes, individuals from these patterns are at increased risk of developing CRC, whose are having a single affected first-degree relative (IE, parent, child, sibling), they are likely increasing to risk of developing CRC (1-7) fold over to normal individuals, personal or family history of sporadic CRCs or adenomatous polyps the

diseases with polyposis include the history of adenomatous polyps, adenomas is leading to increased risk of developing colorectal cancer in these persons, especially the polyps had many numerous ,that have taken a big size and the cases who had colorectal cancer until though it has been completely removed, they have more chance to develop new cancers in other areas of the colon or in rectum, the likelihood of this happening is greater if you had your first colorectal cancer during the stages of young age (Luz, 2008).

1.5. Stages of Colorectal Cancer

1.5.1. Different Stages of Colorectal Tumorigenesis

There are three successive stages to be Tumorigenesis in CRC:

1) Aberrant crypt foci (ACF) are groups of abnormal tube-like glands in the colon and so in the rectum that can be dysplastic or non dysplastic, ACFs are one of the first changes visible in the colon that may lead mostly to cancer (Basel, 2008).

2) Adenoma: Generally Adenomas develop from dysplastic aberrant crypt foci, they commonly called adenomatous polyps. There are many types of adenomas: tubular, tubular-villous, and villous adenomasc

3) Carcinomas: Carcinomas are developing from autonomous and therefore lead to adenocarcinoma these lesions are highly dysplastic and invade the surrounding tissue, the different stages of adenocarcinoma are very important for the diagnosis and main factors for the classifications are the grade of infiltration into the tissue and the presence or absence of metastasis, through the development from a normal colon crypt to a cancer cell additional mutations in oncogenes or in tumor suppressor genes give rise to colonel expansion, (See figure 1.4) it is believed that at least four sequential genetic changes are necessary for colorectal cancer evolution and development. Basic targets for these genetic changes are KRAS, APC, SMAD4 and TP53 (Colussi et al., 2013).

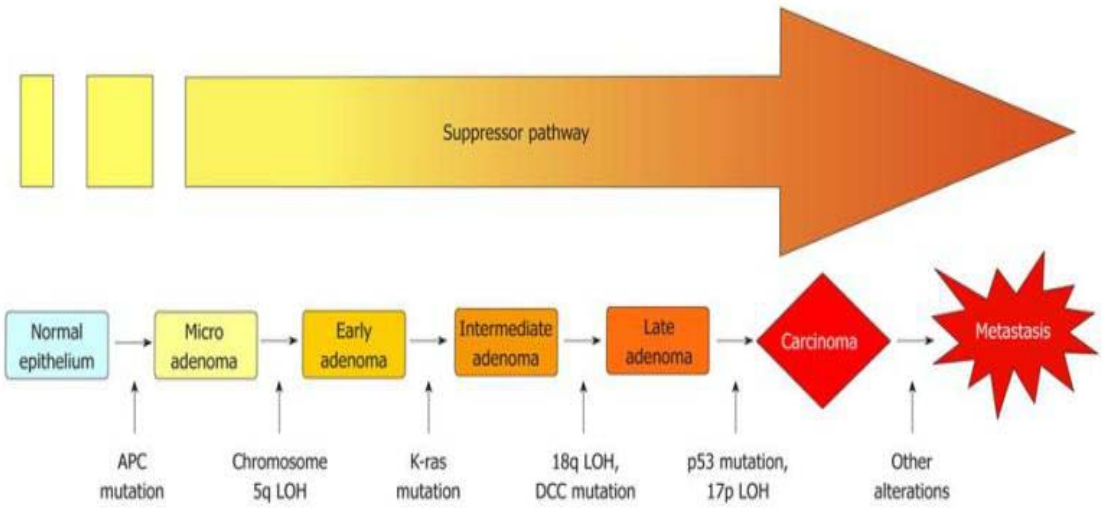


Figure 1.4. Different stages of colorectal tumorigenesis with LOH in TSGs**.** (Moran, 2010)

1.5.2. Stages of Colorectal Cancer

The classification of cancer Cases of colorectal cancer into various stages is used for many reasons to give an indication of diagnosis, to assistance in planning the more effective course of treatment, to aid evaluate the results of treatment and in order to facilitating information exchange and research into cancer. There are many staging systems in use for CRC but there is currently no general consensus on which system is the most appropriate for use, (foster, 2012), The most common systems they use to diagnose it are American Joint Committee on Cancer known also as the TNM system (AJCC/TNM), UICC (International Union Against Cancer), dukes and Astler-Coller staging systems, but the most common staging system used for CRC is American Joint Committee on Cancer (AJCC/TNM). See table 1.1, for correspondences between the three staging systems (Centelles, 2012).

Table 1.1. A comparison of TNM and Dukes classification (Centelles, 2012).

TNM Classification American Joint Commission on Cancer				Classification Ducks stage
Stages	T	N	M	Stages
Stage 0	Tis	N0	M0	
Stage I	T1	N0	M0	A
	T2	N0	M0	B1
Stage II	T3	N0	M0	B2

	T4	N0	M0	B2
Stage III	T1,T2	N1or N2	M0	C1
	T3,T4	N1or N2	M0	C2
Stage IV	AnyT	Any N	M1	D

Primary Tumor (T), TX – primary tumor cannot be assessed, T0 – no evidence of primary tumor, Tis – carcinoma in situ: intraepithelial or invasion of lamina propria, T1 – tumor invades submucosa, T2 – tumor invades muscularis propria, T3 – tumor invades through muscularis propria into subserosa or into, nonperitonealized pericolic or perirectal tissues,T4 – tumor directly invades other organs or structures and/or perforates visceral peritoneum, regional Lymph Nodes (N), NX – regional lymph nodes cannot be assessed, N0 – no regional lymph node metastasis, N1 – metastasis in one to three regional lymph nodes, N2 – metastasis in four or more regional lymph nodes, Distant Metastases (M), MX – distant metastasis cannot be assessed, M0 – no distant metastasis, M1 – distant metastasis (Centelles, 2012)..

1.6. Molecular Pathways Involved in CRC

Recently, our concept of the mechanisms involved in the CRC inception, development became more clear, the most outcomes of researches have shown to present of at least 3 pathways: chromosomal instability (CIN) microsatellite instability (MSI) and CpG island methylator phenotype (CIMP), (Worthley, 2010). The diverse pathways are characterized by featured models of genetic instability, and pathological behavior characteristics (Pancione, 2012). Majority of colorectal cancer follows the chromosomal instability (CIN) pathway, characterized by widespread loss of heterozygosis and gross chromosomal abnormalities, the second comprises about 15% of CRC and is due to derangement of the DNA Mismatch Repair (MMR) system and consequential microsatellite instability (MSI), As is well a known the Mismatch Repair system and (MMR) it's responsible for the production of proteins which recognize and direct repair of single nucleotide mismatches at microsatellite sequences that escape the proofreading system of DNA polymerase (Colussi et al., 2013). At present, it has been proved that other systems and pathways are involved in, for example, abnormal DNA methylation and newly the discovery of microRNA (miRNA) can play a role to contribute to the carcinogenic process (Pancione et al., 2013).

1.6.1. Chromosomal Instability (CIN)

The most well featuring types of colorectal pathway are chromosomal instability (CIN), tumorigenic process includes different mitotic spindle checkpoint regulators and proteins which mutually influence mitotic chromosome stability (Colussi et al, 2013). The CIN pathway is linked directly with the sequential deregulation of tumor suppressor genes (TSGs) and oncogenes such as APC, KRAS, DCC/SMAD4, and TP53, it mostly occurs within inherited tumors like familial adenomatous polyposis (FAP), As well as, it has seen associated with the majority of sporadic CRCs too (Pancione et al, 2012). There is disagreement exists as to whether genomic instability begins the adenoma-carcinoma sequence APC or whether it develops during the process and supports growth to CRC. Chromosomal instability or microsatellite instability had been observed in adenomas cells. Thus, genetic instability can show to be present during the inception of adenoma, before APC gene mutation and progression to outright malignancy (Colussi et al, 2013).

1.6.1.1. The WNT Signaling Pathway

Adenomatous polyposis coli gene (APC) related with situation of polyposis and include Familial Adenomatous Polyposis (FAP), attenuated FAP (AFAP), Gardner and Turcot syndromes, as a known ,the APC of Wnt/ β-catenin pathway leads to carcinogenesis in both sporadic and hereditary CRC. approximately (98%) of APC mutations are either frame shift or nonsense mutations cause to resulting in truncated protein, this mutation shown in about 30%–70% of general sporadic adenomas and sporadic colerectal cancer. (Armaghany et al, 2012). During the cell cycle in G1 to S phase normally the (APC) tumor suppressor gene block transition, the Wnt signaling pathway plays role to stay native stem cells in their undifferentiated state in the base of the colonic crypts and also maintains to the survival of cancer cell stem cells, β-Catenin has an active Instrumental in the Wnt signalling pathway, unmutated APC leads degradation of β-catenin and thus functions as a negative regulator of the Wnt signaling pathway. Sustained levels of intracellular β-catenin result in a long period activation of the Wnt pathway in APC mutated colorectal cancer cells (Armaghany et al, 2012) (Cloussi et al 2013). When APC is mutated β-catenin degradation complex does not take form, and β-catenin accumulates in the nucleus. β-catenin is able to bind to the T-cell factor (TCF) family of transcription factors which again activate

transcription of other tumor promoting genes, such as CMYC, and cyclin D1 (CCND1) (Khan et al., 2011). Wnt signalling pathway may be disrupted also by mutated β-catenin. About 50% the tumors with wild type APC display mutations in β-catenin encoding gene CTNNB1, suggesting that CTNNB1 mutation can substitute APC mutation in colon carcinogenesis, However, the mutations in APC and CTNNB1 are not mismatched through functionality, since adenomas with CTNNB1 mutations seem not to progress to malignant tumors as far as adenomas with APC mutation (Laiho, 2005).

1.6.1.2. K-RAS

The K-ras gene encodes for a plasma membrane-bound GTP binding protein with a molecular weight of codon 21(Al-allawi et al., 2012). This protein is responsible for many pathways of cell such as growth, differentiation, and apoptosis , mostly of mutations in the K-ras gene happen at codon 12 and at codon 13 while Mutations at codon 12 grants a more oncogenic phenotype of the mutations at codon 13 (Colussi, 2013). Moreover The RAS plays a role to effect critical for the emergence of carcinogenesis, results of Scientists proved that high WNT activity has been associated with increased MAPK signaling, in K-ras mutated colorectal cancer patients. In addition, many studies found an interaction between the AMP-activated protein kinas (AMPK) and MAPK. AMPK is a cellular energy balance status sensor, and stimulate to regulation cell proliferation and development through the inhibition of the mTOR pathway and activation of the CDKN1A (p21) pathway and p53.

1.6.1.3. TP53

The TP53 gene is significantly involved in the control of the cell cycle, p53 loss of function is often present in the later stages of CRC, p53 protein induces G1 in cell-cycle arrest, and facilitates DNA repair prior to a cell committing to the process of DNA replication (Armaghany et al., 2012), owing to various functions is known as ‘the guardian of the genome’ which be responsible, essentially to the cellular gatekeeper of growth and division during cell cycle, in chromosome 17p13.1 region the p53 gene contains 11 exons and transcribes a 2.8 KB MRNA which is translated into a 53 kDa protein, p53, a 393 amino acid long phosphoprotein, acts as a key

regulator of cell cycle (Kkan et al., 2011). Studies noted when p53 is mutated, the protective role of WAR-1 is nonexistent, cyclin- moreover the p53 has affect regulate energy balance during activation of the AMPK pathway (Colussi et al., 2013). During the evolution of CRC disease mutations in different dependent kinases (CDKs) are also involved P53 through the AMPK pathway up-regulates the CDK inhibitor 1A (CDKN1A or p21) which is involved in regulating the cell cycle Phases (Khan et al., 2011). Another function, P53 is to interact with Cyclooxygenase-2 (COX-2), which acts a role in promoting inflammation and cell proliferation in the CRC, importantly COX-2-positive tumors were found to be associated with an increased cancer-specific mortality regardless of p53 status, Pointing out that COX-2 could be an independent prognostic factor of CRC (Colussi et al., 2013**)**. An often co-occurring molecular alteration with the p53 loss is the loss of heterozygosity of chromosome 18q about (65.4%), frequetly, the remaining allele is affected by a mutation (Armaghany et al., 2012). The DCC (Deleted in Colorectal Carcinoma) gene is located on the long arm of chromosome 18 (18q21.3), it encodes the transmembrane protein DCC, the DCC is a “conditional tumor suppressor gene”, (see figure 1.6), (Armaghanyet al., 2012).

Other Pathways Involved in CIN, PIK3 represents a family of lipid kinases that have a role to affect signaling pathways for regulating in some of the cell functions during processes such as cell proliferation, survival, motility adhesion and differentiation. About 40% colorectal cancers is up-regulated by PIK3 signaling pathway, PIK3 have role limited at a pre-malignant colorectal tumors therefore, the effect just before coincident with invasion the cancer (Cederquist**,** 2005).

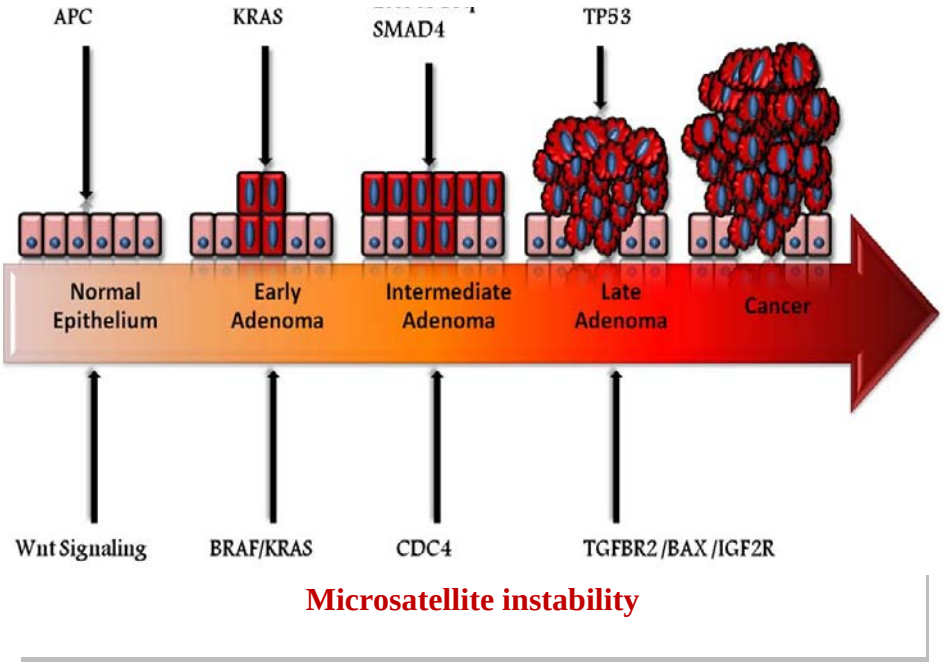


Figure1.5. Mechanism of chromosome instability and microsatellite instability (Moran, 2010)

1.6.2. Microsatellite instability (MSI)

MSI is another pathway of tumorigenesis of CRC, approximately 15% cases of all colorectal cancer, MSI in tumorigenesis is leading the inactivation of mismatch repair (MMR) genes, MMR is a system which can recognize of repairing erroneous insertion, insertion/deletion loops caused by slippage of DNA polymers as well as repairing some forms of DNA damage, in addition to its ability of repairs mismatched nucleotides. MSI occurs in hereditary as well as sporadic CRC, a germline mutation in an MMR gene reaches up by about 90% of cases (Laiho et al., 2010). Some of the identified key genes in MSI colorectal cancer that contain such sequences include TGFßRII, BAX, and IGFIIR, sporadic MSI colorectal cancer is commonly caused by promoter hypermethylation of the MMR gene MLH1 resulting in the inactivation of this gene, The familial form of MSI CRC is (HNPCC & lynch syndrome) CRC, which is caused by germline mutations in tumor genes MLH1,

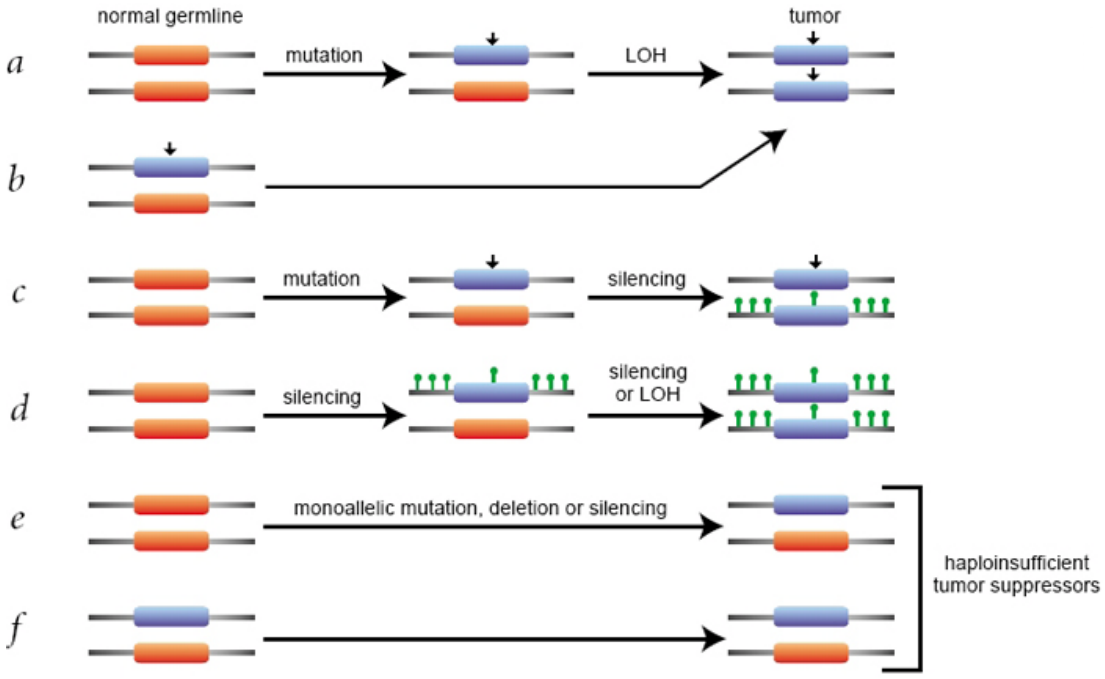
PMS2, MSH6, or MSH2, and accounts for about 3% of all CRC cases, see figure 1.5, (Colussi et al., 2013)

1.6.3. CIMP and the "Serrated" Pathway

The connotation of CpG island metylator phenotype, or CIMP, it was known a compel and somewhat controversial for scientists, the CIMP refers to the concept that a sub of particles of tumors has widely methylation of CpG islands that causes to epigenetic Inhibition of tumor suppressor genes by promoter methylation(Wad and Samowitz., 2007). About 20-30% of sum CRC it might be caused by CIMP Depending on reports, clinical features of CIMP from patients CRC linked with MSI, also they saw the relationship between CIMP and histological grates was the silencing of the p16INK4a tumor suppressor gene which loss of function gene led to uncontrolled cell proliferation, then turn into neoplasm (Colussi et al., 2013)

1.7 Loss of Heterozygosity (LOH)

Increased frequency of chromosomal deletions affected as LOH is a hallmark of genomic instability in cancer. when tumor suppressor genes lose a Functional lead to LOH at specific loci during colonel selection for growth advantage define the minimally lost regions potential of their areas on chromosomes, loss of heterozygosity has affected at the molecular or cytogenetic level as a deletion, a gene conversion single or double homologous and no homologous mitotic recombination, a translocation, chromosome breakage and loss, chromosomal fusion or telemetric end-to-end fusions, or whole chromosome loss with or without accompanying duplication of the retained chromosome (Hankey and Groden, 2013), we tried explain models Loss of TSGs function in cancer and discuss each case by figure (See figure 1.6).



Figur 1.6. Loss of TSGs function in cancer (Balmain, 2003)

In above figure (a, b) the classical knudson two-hit model involves an initial mutational event vertical arrowhead which leads to gene inactivation during tumor progression each blue bars indicate inactivate genes, loss of heterozygosity by non-disjunction,mitotic recombination or deletion results in the functional inactivation of both alleles, if the first mutation (deletion) is inherited through the germline individuals carrying this mutation are often highly predisposed and chance to tumor development. in (c),the mutational event can be followed by gene silencing through promoter methylation without loss of heterozygosity while in d biallelic silencing both of copies gene without LOH or gene mutation and in (e, f) haploinsufficient TSGs do not need to lose both functional copies to give more risk ,loss of a single gene copy may appear by mutation ,deletion or silencing and the other functional allele may be retained, in some example in (f) ,a partially or completely nonfunctional allele may be inherited through the germline, predispose an individual to tumor progression without requiring loss of heterozygosity or complete functional inactivation .some low -penetrance tumor susceptibility genes may be in this category (Balmain et al.2003)

1.7.1 Microsatellite Analysis and Loss of Heterozygosity

Microsatellites may be define are noncoding tandem repeats of one to six nucleotides deployed on along genome. Microsatellite sequences of an individual are constant for life and are match in every tissue. the microsatellites on the two or three alleles are slightly different in most individuals, which are indicated to as heterozygosity (Bibbo, 2009). Deletion of one of the alleles leads to loss of heterozygosity (LOH). The procedure includes PCR amplification and locus-specific microsatellite analysis, which based microsatellite polymorphisms have demonstrated their ability in genetic linkage analysis and other identification methods, due to their high the content of information and even distribution over chromosomes (Nakamura, 2009) Microsatellites linked to tumor suppressor genes or related tumor genes involved in malignant transformation are most often analyzed, Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, commonly N, N'-methylenebisacrylamide, acrylamide (PAGE) is a well-established usually used method for detection of heterozygous samples and LOH ,The analysis of polymorphic microsatellite markers in acrylamide gel should appear just one (homozygous) or two (heterozygous) major bends, which supports alleles through analyzing genes(re-peated DNA sequences around the gene) (Korolija, 2008).

CHAPTER 2

LITERATURE ABSTRACTS

Khan and his colleagues (2011), they were studying aim to determine the ratio frequency of loss of heterozygosity of gene DCC in CRC cases, collected from 80 patients, they used two markers one within the polymorphic locus D18S8 localized to 18q21.3 and the other in VNTR, loss of heterozygosity in DCC gene was observed in (32.5%) at VNTR and (23.75%) at D18S8- M2 region in CRC cases. LOH of the DCC gene of two markers Calculated to 56.25 % of CRC patients.

Eighty-three CRC patients were analyzed by Polymorphic microsatellite markers to a chromosome 20 in patients with sporadic colorectal cancer tumor & normal DNA they did it by Peng &his colleagues (2002), the average loss of heterozygosity frequency was As follows: 21.1% was on the long arm 20q , 26.7% was the on short arm 20q and 22.8% was on the whole chromosome

In 2003 Laiho his colleagues they known already relationship between chromosome 22 in regions 13 and aconitase 2 (ACO2) in many of CRC patients when it lost this regions, 203 CRC samples showing a deletion in chromosome 22q13 were sequenced for mutations in ACO2.they saw in results ,that ACO2 is often deleted in colorectal cancer but is unlikely to be the true target of the deletions because they noticed two silent polymorphisms in exons 3 and 5, but did not detect any pathogenic mutations.

Ozaslan and Aytekin in 2009 They concluded from their research LOH of tumor suppressor genes from colorectal cancer patients had seen at various loci on different chromosomes like 1p, 1q, 4q, 5q, 8p, 9q, 11q, 12p, 14q, 15q, 17p, 17q, 18p, 18q and q22, In addition, the linkage had between tumor suppressor genes and initiation development CRC through development CRC through loss of its (TSGs).

Analyzing eighty colorectal cancer cases to MSI status and LOH at chromosomes 5q21, 8p12-22,17p13, and 18q21, that get by whoever Matsuzaki and his colleagues in 2005 the results reached 14 cases of MSI (17.5%) and 58 cases of LOH (72.5%), loss of heterozygosity was showed more frequently in microsatellite stable (MSS) CRCs than in microsatellite instability (MSI) CRCs at all loci.

Zauber his colleagues (2008) they were analyzed 110 colorectal carcinomas cases and adenoma the deletion in colorectal cancer (DCC) gene and SMAD4 gene to signalize the participation of this DNA segment of chromosome 18q in colorectal carcinogenesis , they used two marker for (DCC and SMAD4) as for LOH fond five marker for 23 lesions with inconsistent results. The results for loss of heterozygosity of DCC and SMAD4 were identical in molecular analysis for 122 (79.7%) of 153 informative samples. The physical proximity of genes DCC and SMAD4 will usually be done, but not fixed, the outcome in similar loss of heterozygosity findings.

In 2001 Thiagalingam his colleagues, they were a detailed investigation of the 5 chromosomes delete most frequently in human colorectal cancers. A total number of 10,216 selections were made with 88 microsatellite markers, revealing 245 chromosomal loss events. The mechanisms of loss were marked chromosome-specific, more than 50% of the losses were associated with losses of only part of a chromosome rather than a whole chromosome.

In a study of Ezak & his colleagues in 2003 selected random 35 microsatellite markers, and execution allelotype study in colorectal cancer patients or dysplastic lesions from ulcerative colitis. High frequency of loss of heterozygosity (62.5%) was detected on chromosome 6 (D6S468) but not on other chromosomes, in addition, they used four microsatellite markers around the D6S468 locus ,the analysis were Resulted in the commonly deleted region between two loci, D6S1543 and D6S1580 more than other . Surprisingly, there was no LOH in this region in sporadic CRCs and severely inflamed lesions of longstanding and extensive UC without cancers. These results indicated the presence of novel tumor suppressor genes on chromosome 6 related to the carcinogenesis of ulcerative colitis but not to sporadic CRCs.

Takahashi and his colleagues (2003) Therefore, their examined the condition of MSI and loss of heterozygosity (LOH) of UC epithelium using a crypt isolation technique Methods. 129 biopsy samples from 21 patients with UC were investigated for histology and microsatellite status, using 9 microsatellite markers. A total of 1031 polymerase reaction chain (PCR) products were evaluated, they showed Results insignificant with microsatellite markers displayed instability, while in LOH at the 3p locus was detected in the nondysplastic epithelium of one patient with longstanding UC.

Aytekin and his colleagues did research in 2010 and their used 5 microsatellite markers to analyze the region 12q13-24 among Forty-seven cases with CRC. The frequency of loss of heterozygosity and the clinicopathological data were compared using logistic regression and a chi-square test. In 34 of 47 tumor tissues (72%), LOH was detected at least in one marker. The highest percentage LOH frequency was 34%, on the D12S129 loci, the lowest frequency was 23%, on the D12S78 loci. Loss of heterozygosity was detected as 32% on D12S83, 30% on D12S346, and 26% on D12S1660.In addition, that insignificant relation was found between the frequency of LOH and clinic pathological Characteristics ($P > 0.05$).

Wan and his colleagues in 2006, they study about loss of heterozygosity (LOH) on 12p12-13 in Chinese colon carcinoma patients ,in research used ten specimens of cancer tissue, ten specimens of adjacent tissue and ten specimens of normal tissue by eleven microsatellite markers on 12p-12-13. (PCR) and denaturing polyacrylamide gel technicality were used, mostly of LOH was found on D12S1034 & D12S1591 in 42.86% (3/7) of carcinoma tissue specimens. The incidence of LOH did not relate with sex, age, tumor size and lymph node metastasis. They Concluded Genomic instability may get on 12p-12-13 of K ras 2 genes in the progression of CRC.

Luo and his colleagues in 2006 ,they select 9 microsatellite markers close to specific genes, that was at APC (5q21), PTPRJ (11p11), p53 (17p13) and DCC (18q21) they were analyzed in 32 ACF and at normal samples crypts from the same 28 cases . 6 LOH found were in five of 32 ACF: four (LOH) were at 11p11, the results of LOH in ACF with normal expressions of (APC) and beta-catenin proteins suggests that LOH can incidence quickly in colon neoplasia and maybe before APC deletion the showing of 3 of 4 of LOH at 11p11 for PTPRJ and half of all the LOH in this study

at PTPRJ suggest that this gene had an effective role in for beginning colon neoplasia.

In 2011 each of Maleno & his colleagues studied the frequency of LOH-15q21 in tumor tissues randomly, by identifying the status of heterozygosity of 2 microsatellite markers we detected LOH-15q21 in 44% of bladder carcinomas (n = 69), in 35% of colon carcinomas (n = 95), in 16% of melanomas (n = 70) but only in 7% of renal cancers (n = 45).fortuitously, they noted a frequent of LOH-15q21 and LOH-6p21 in colorectal carcinoma, bladder carcinoma and melanoma, while in renal carcinoma did not affected , that the high Occurrence of LOH-15q21 in some tumors and the Occurrence of LOH-15q21 and LOH-6p21 have strong role impact on tumor immunogenicity and on the efficiency of cancer immunotherapy.

SW and his colleagues (2002), they were comparatively analyzed using a reference of forty microsatellite markers in 8 cancer-related chromosomes, 3p, 4p, 5q, 8p, 9p, 13q, 17p, and 18q Of the 168 CRC analyzed, 29 (17%) with high-frequency MSI(survival $P < 0.05$). while of LOH detected in 139 (83%) cases, High- range of loss linked with earlier onset, lymphatic invasion, and rectal location, however low-level loss was more common in proximal colon and stages I and II ($P < 0.05$). Their Believed The classification of colorectal cancer depended on chromosomal loss and MSI offers a prognostic index that reflects tumor pathobiology.

In study of SF and his colleagues in 2008 they used 83 cases from CRC (normal and cancer), which were tested in 16 polymorphic microsatellite markers were analyzed on chromosome 5 and another six markers on chromosome 5p15 , in this study the PCR Technique was used . Products PCR were electrophoresis on an ABI 377 DNA sequence. Gene scan 3.1 and Genotype 2.1 software were used for LOH scanning and analysis. 2 distinct regions of frequent allelic deletions at D5S416 on 5p15 and D5S428-D5S410 on 5q were detected. While 6 polymorphic microsatellite markers were applied to 5p15 and the lower region of frequent LOH was founded on 5p15 spanning the D5S416 locus. Specific location of 5p deletions in region 5p15.2-5p15.3 had detected, Probably that contained one or more unknown TSGs connected to CRC in that region.

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

This study was approved by the Gaziantep University Clinical Research Ethic Committee (30.040230-172). Archival formalin-fixed, paraffin-embedded tissues from 42 colorectal cancer and normal samples were obtained from the Central Laboratory of Pathology at Duhok - the Kurdistan region. We started to collect paraffin embed tissues during May 2013 and ended in July 2013.

3.1.1. Chemicals and Devices For DNA Isolation From Paraffin Embed Tissues

- Freeezer (- 20 ^{0} C) (Vestel, Turkey)
- Spectrofotometer (Thermo Scientific ,America)
- Sensitive balance (sensitive, Switzerland)
- Shaker
- Vortex (Velp Scientific, Italy)
- Centrifuge (Hettich,Germany)
- Micropipettes (1-10µl, 10-100µl ve 100-1000µl) (Eppendorf, Germany)
- Ethanol (%96) (J.T. Baker, America)

DNA isolation kit (*Geneaid*, USA)

3.1.2. Chemicals and Devices for PCR Amplification and Agarose Gel Electrophoresis

- Thermal Cycler (Takara, Japan)
- Horizontal gel electrophoresis (Cleaver, England)
- UV transilluminator (Bio-Rad, Germany)

- Vortex (Velp Scientifica, Italy)
- Microwave oven (Archelik, Turkey)
- Micropipettes (1-10µl, 10-100µl and 100-1000µl) (Eppendorf, Germany)
- Taq PCR Master Mix Kit(QIAGEN ,German)
- −20°C Laptop cooler (Nunc / Thermo Scientific, America)
- 10X buffer (New England Biolabs, America)
- $MgCl_2$ (New England Biolabs, America)
- dNTPs (Vivantis, America)
- Taq DNA polymerase (New England Biolabs, America)
- Forward and reverse primers (Alpha DNA, Canada)
- Formamide (Sigma, America)
- d.d H_2O (İdol, Turkey)
- Agarose (peqGOLD, America)
- Ethidium bromide (EtBr) (Merck, America)
- 10X TBE Buffer:
    - Tris (Amresco, America) ……..…….108 g
    - Boric acid (Scharlau, Spain)…..…….55 g
    - EDTA (Merck, Germany) ……..9.37 g
    - Solve in 1000 ml distilled water and adjust pH at 8.4. Autoclaved and store at $+4^{0}$ C.
- Pipette tips (10, 100 and 1000 µl) (Gilson, America)
- PCR tubes (0,2 and 0,5 µl) (Oxygen, America)
- 6X Loading buffer (Sambrook and Russel, 2001):
    - Bromophenol blue (Merck, America) ……...……%0.025
    - Xylene cyanol FF(ABCR, Germany) ……...……%0.025
    - Sucrose (Fluka, America) …..……...%40
- DNA Ladder- 50 bp (Thermo Scientific, America)

3.1.3. Chemicals and Devices for Polyacrylamide Gel Electrophoresis

- Manual DNA Sequencer (Sequi-Gen GT Electrophoresis System, Bio-Rad, Germany)
- Power Supply (Bio-Rad, Germany)
- Sensitive balance (Precisa, Switzerland)
- Thermal cycler (Takara, Japan)
- Vortex (Velp Scientifica, İtaly)
- Centrifuge (Hettich, Germany)
- Magnetic stirrer (heat) (Lab. Companion, Korea)
- Ultrasonic sonicator (Selecta, Spain)
- Micropipette (1-10µl, 10-100µl ve 100-1000µl) (Eppendorf,Germany)
- Acrylamide stock solution (%30):

  Acrylamide (Roth, Germany) ….....29 g
  Bis acrylamide (Vivantis, America)...1g
  Distiled water……...……100 ml
- Urea (8M) (Sigma, America)
- TEMED (Roth,Germany)
- Formamide (Sigma, America)
- APS fresh solution (10%):

  Ammonium persulfate (ABCR, Germany)…0.10 g
  Distilled water…….....1 ml
- Dimetildiclorosil (Sigma, USA)
- Adhesion solution:
  - Acetic acid (%99) (Sigma, America)…….. 25µl
  - Metakriloksipropiltrimetoksilan(Merck, Almanya)……. .15 µl
  - Absolut ethanol (J.T. Baker, America) ….…….…… .5 ml
- 10X TBE buffer
- 6X loading buffer:
  - bromophenol blue (Merck, America)..……%0,01
  - Ksilen siyanol FF(ABCR,Germany)…….%0.01

- EDTA (Merck,Germany) ……...20 mM
- Formamide (Sigma, America)……..……...%80

- PCR tubes (0,2 µl, lik) (Axygen, America)
- Pipette tips (10 ve 100 µl' lik) (Gilson, America)

3.1.4. Chemicals and Devices For Silver Nitrate Staining

- Shaker (Selecta, spanish)
- Silver nitrate (Merck, America)
- Absolute ethanol (J.T. Baker, America)
- Sodium hydroxide (Tekkim, Turkey)
- Formaldehyde (%37) (Tekkim, Turkey)
- Acetic acid (%99) (Sigma, America)
- Digital camera (Sony, Japan)
- Light box (Seica, America)
- Glass Slides (40x60 cm)

3.2. METHODS

3.2.1. DNA Isolation

DNA of CRCs were extracted from the embedded paraffin tissues , the tissues were washing several times in xylene to remove the wax , then the xylene is treatment by performing multiple washes with ethanol before DNA isolation. DNA isolated by a kit (Geneaid kit USA), at first transferred up to 25 mg of tissue to 1.5 ml microcentrifuge tube, add 200 µl of GST Buffer and 20 µl of proteinase K then vortex thoroughly. Incubate at 60ºC overnight until had been clear completely ,then centrifuged for 2 min at 14-16,000 x g, slowly , transferred the supernatant to a new 1.5 ml microcentrifuge tube, then we added 200 µl of GSB Buffer then shacken vigorously for 10 seconds, added 200 µl of absolute ethanol to the sample lysate and mixed at once by shaking vigorously for ten seconds, put GD Column in 2 ml collection Tube transferred all of the mixture to the GD Column, Centrifuged at 14-16,000 x g for one minute, discarded the 2 ml Collection then transfer the GD

Column to a new one of 2 ml Collection tube, added 400 µl of W1 Buffer to the GD Column. Centrifuged at 14-16,000 x g for 30 seconds, discarded the flow-through. Placed the GD column back in the 2 ml collection tube. Added 600 µl of wash buffer to the GD column. Centrifuged at 14-16,000 x g for 30 seconds then discarded the flow-through. Put the GD Column back in the 2 ml Collection Tube. Centrifuged again for 3 minutes at 14-16,000 x g to dry the column matrix, Standard elution volume added 100 µl. Transferred the dried GD Column to a sterile 1.5 ml (microcentrifuge tube). Add 100 µl of Elution Buffer (incubate at water bath 60 C) into the center of the column matrix. Let stand for at least three minutes to allowed Elution Buffer, to be completely absorbed. Finally centrifuged at 14-16,000 x g for 30 seconds to elute the purified DNA.

After Isolation step, the DNA samples were performed spectrophotometrically 260/320 nm, to determine measurement of the concentration quantitatively of the DNA samples , purification DNA was done which samples need it , manual procedure was used (ethanol & NaCl) to purified DNA .

3.2.2. PCR Amplification and Agarose Gel Electrophoresis

DNA isolation amplification of DNA samples were done by Technique polymerase chain reaction (PCR). The polymerase chain reaction (PCR) is a most important scientific technique in molecular biology to amplify and increasing a single copies of a piece of DNA after crossing at a several orders of magnitude, thus reproduce sequences between thousands to millions of copies of a part purposeful DNA . Three major steps has involved in the PCR technique: Denaturation, annealing, and extension. 5 microsatellite markers amplified with each 42 sample (normal and tumor). In this study we use both methods for amplification reaction manual method and kit (QIAGEN, Germany) (See table 3.2 and Table 3.3), polymorphic microsatellite markers were obtained from NCBI data base (www.ncbi.nlm.nih.gov). One of DNA from CRC samples were used to determine the gradient PCR for each primer pair. Amplification were carried out in an AB thermocycler. Depending on the result of agarose gel electrophoresis, the optimum melting temperature of all primers were determined for fixing annealing temperature for each markers, so base sequences and base pairs were given on (Table 3.1).

Table 3.1. Microsatellite markers show sizes, base sequence and regions

Primer name		**Sequence of Primers**	**Molecular weight**	**The Region**
D21S270	FP	GAAATGTTTTAATAAATGGTGGTTA	199-223 bp	21q22.13
	RP	ACAAAGTTATGGTCAAGGGG		
D21S1981	FP	GATGGACATGCTGCACTCTATG	195 bp	21q22.3
	RP	GAACCCAAAACATTCTTGCAGCC		
D21S1440	FP	GAGTTTGAAAATAAAGTGTTCTGC	157-175 bp	21q22.1
	RP	CCCCACCCCTTTTAGTTTTA		
D21S1839	FP	GAGGGGACTGATTCCTAGAGG	152-153 bp	21q11.2
	RP	AGAATGGGCCTTGCTTTTTC		
D21S303	FP	GATGGCTCTGCATTTCTATG	256 bpr	21q22.12
	RP	CCTGCTAAATTAGAGCTGCA		

Table 3.2. PCR reagents and their concentrations from kit

Reagents prepare from kit	**Conc.**
Master mix : MgCl2, 25 mM 10 xbuffer Taq DNA Polymerase dNTP mix	 **15 mM** **-** **250 units** **200 µM of each one**
Distilled water	**-**
Primer FP & Primer RP	**0.1–0.5 µl**
DNA sample	**≤1 µg/reaction**

Table 3.3. PCR reagents and their concentrations

Reagents	Concentration
Distilled water	-
10X buffer	1X
$MgCl_2$ (25 mM)	1-4
dNTP mix (2 mM)	0.2 µl
Primer FP	0.1-1 µl
Primer RP	0.1-1 µl
Taq polymerase (0,5 U)	1,25 u/50µl
Formamide	1 µl
DNA sample	10 pg-1 µg/50 µl

The thermocycling program was set to run 35 cycles according to the following parameters as shown in table 3.4

Table 3.4. Identified optimal PCR conditions for primers

Temperature ^{0}C	Time	Number of cycles
94	5 min	35X
94	45 sec.	
55-70*	45 sec.	
72	45 sec.	
72	8 min	
4	∞	Hold

3.2.3.1. Agarose Gel Electrophoresis

The final products were analyzed by Agarose gel, (agarose gel electrophoresis is used to test the efficiency of PCR reactions). Each samples was running in (1.3 -2%) agarose gel so it were stained with a compound that makes the DNA band visible under UV light. Ethidium bromide is routinely utilized to stain DNA in agarose gel. Samples were electrophoresed under electric field of 110 volts up to 40 minutes. The length of the PCR products ranges between 150 -270 bp.

3.2.3.2. Polyacrylamide Gel Electrophoresis (PAGE)

Acrylamide (PAGE) is a well-established usually used method for detection of heterozygous samples and LOH**,** the reaction is a free radical polymerization, always, carried out with ammonium per sulfate as the initiator and N,N,N',N'-tetramethylethylendiamine (TEMED) act as activator (Korolija, 2008)**.** The percentage of gel were prepared after molecular weight of primers have been obtained from samples when running on an agarose gel revealed. The presence of DNA sequences for the detection of PCR products analyzed by (Sequi-Gen GT Electrophoresis System, Bio-Rad) were carried out in denaturing urea polyacrylamide gel and running samples to detect samples and calculated LOH (Korolija, 2008).

3.2.3 Preparation of Glasses

The U-shaped and rectangle shaped glasses were cleaned before using by ethanol (70%) and the process were repeated three times and was left for 10 minutes to dry. The spacers were placed in the two the end sides and bottom of basic glass, then put the other glass on it and carefully, then closed by the catcher to fixing and avoiding movement the glasses.

3.2.4. Preparation the Gel and Loading the Samples:

We prepared acrylamide gel (7%) from 36 g urea, 15 ml of distilled water, 32 ml of formaldehyde, 10 ml of 10XTB and 17.5 ml acrylamide (bisacrylamide (29:1) from a stock solution by adding a total volume of 75 ml was completed) then mixed well to solve chemical substances. Then 1 ml of freshly prepared stock solution of APS 10% and 40 µl TEMED was added quickly, The gel must be poured between the glasses carefully to avoiding any air bubble formation. Shark-toothed comb was put on the top of the gel and it was left, at least for 1-2 hour at room temperature for

polymerization. We removed a comb, at same time we tried to cleaning a tops of both a glasses from increase solid gel, clean it from all latches, then the glasses were fixed in the trunk of the electrophoresis apparatus. The upper and lower part of the tank was filled with 1X TBE. The teeth of the comb were put carefully in the top of the gel between two glasses to form wells for loading samples. Power supplier was applied at 80 volts for 45 minutes for pre running in order to prepare it to work. 5 µl PCR product and 5µl loading dye were mixed and denatured at 95 -98ºC for 5 min and then at the time placed into ice for 5 min. 4 µl of each diluted sample were loaded into the wells of polyacrylamide gel. Electrophoresis was performed by using a vertical electrophoresis system about 3 hours.

3.2.5. Staining of Samples on the PAGE Gel

After the time running gel electrophoresis was finished, we tried removing glass rectangle which has contain gel and we started to staining it. In all steps were used about 1.5 - 2 liter, we performed on shaking (see the Table 3.5). After finished all steps and the gel were let to dry, we showed at the box-light, the DNA bands were photographed and then ready for analyzed.

Table 3.5 Steps and chemical solutions used for staining acrylamide gel

Steps	**Solutions**	**Time**
Fixation	10% absolute ethanol, 0.5% acetic acid (per one liter)	5 min
Soaking	1.5 g of silver nitrate, 1.5 ml of formaldehyde (per one liter)	6-7 min
Washing	Distilled water	10-15 Sec.
Development	15 g of sodium hydroxide, 2 ml of formaldehyde (per one liter)	3-5 min
Stop	10% of absolute ethanol, 0.5% acetic acid (per one liter)	2 min

4.1. Statistical Analysis of Datas

Statistical Analysis of Data Statistical analyzed using the program GraphPad Instat (version 3.05) was performed. Results were expressed as the standard deviation or percentage. Statistical significance at $p < 0.05$ was taken, the incidence of loss of heterozygosity, stage disease and age patients with Fisher's exact test was used for evaluation.

CHAPTER 4

RESULTS

4.1. Average Gender of Patients CRC

Between May to July 2013 in Duhok Laboratory Central Pathology colorectal cancer was collected samples and diagnosed. Number of females were 21 and male were 21, (n=42).

4.2. Average Age Patients and Ducks Stages of CRC

The highest age was 82 and youngest age was 19. Average of patients ages was 54, 55 ±17,30 .While patients ≤ 60 were 21, and > 60 were 21. Ducks stages were 23 ducks A&B and 19 ducks C&D (n=42).

4.3. DNA Concentration

DNA concentrations were detected by spectrophotometrically after isolation samples (42 tumor and 42 normal tissue). The highest concentration of DNA was 124.5 nag/µl in tumor samples. In normal tissue, the highest concentration was 73 nag/µl. The lowest concentration of DNA was 4.5 nag/µl in tumor tissue, and the lowest concentration was 5.1 nag/µl in normal tissue.

4.4. Results of PCR Amplification

after were amplified the DNA samples with all microsatellite markers and the products has been running to agarose gel in concentration (1.3-2%).Therefore, we were able to register analysis through that were exposing the gel at UV light . Each samples of DNA normal with tumor were running to next to each other (tumor, normal) at along gel with marker 50 bp (See figure 4.4).

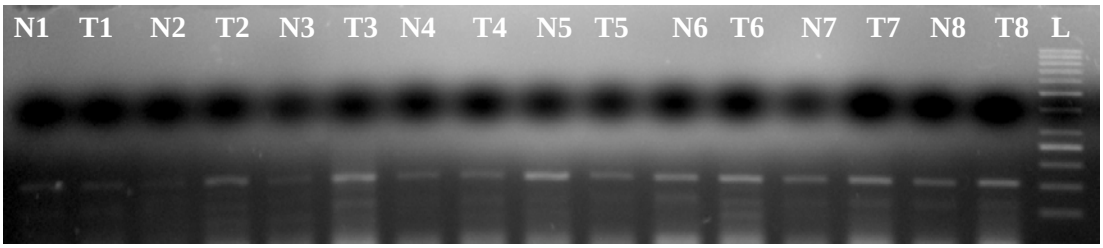


Figure 4.1. Show PCR products amplification of agarose gel electrophoresis.

In 110 V was running it about 40 min at UV viewer. Image with marker 50 bp, (Tx) number tumor samples, (Nx) number of normal samples.

All of the amplifications samples for each microsatellite marker D21S1389, D21S1440, D21S1981, D21S270 and D21S303 were obtained after especial bends had appeared for target marker which was used from tumor samples and normal samples which were running samples (tumor and normal) respectively in agarose gel, thus we obtained all PCR products of samples to be ready for a PAGE.

4.5. PAGE Results (MSS)

All PCR products of the microsatellite markers which electrophoresis at (7% -12%) polyacrylamide gel (PAGE) were detected under static conditions. The percentage of polymorphic in each primer were obtained, different ratio of loss of heterozygosity (LOH), rotational of heterozygosity (RLOH), microsatellite instability (MSI) and non-informative (NI) through light box viewer.

4.5.1. Results of microsatellite marker D21S1389

D21S1839 microsatellite marker at region 21q11.2 which molecular weight 152-153 bp, we used polyacrylamide gel at concentration (12%), we get a clear results the highest frequency of LOH was obtained at this marker 8 (19%), 34 (81%) RLOH from sum 42 CRC sample, see figure 4.2, and Table 4.1.

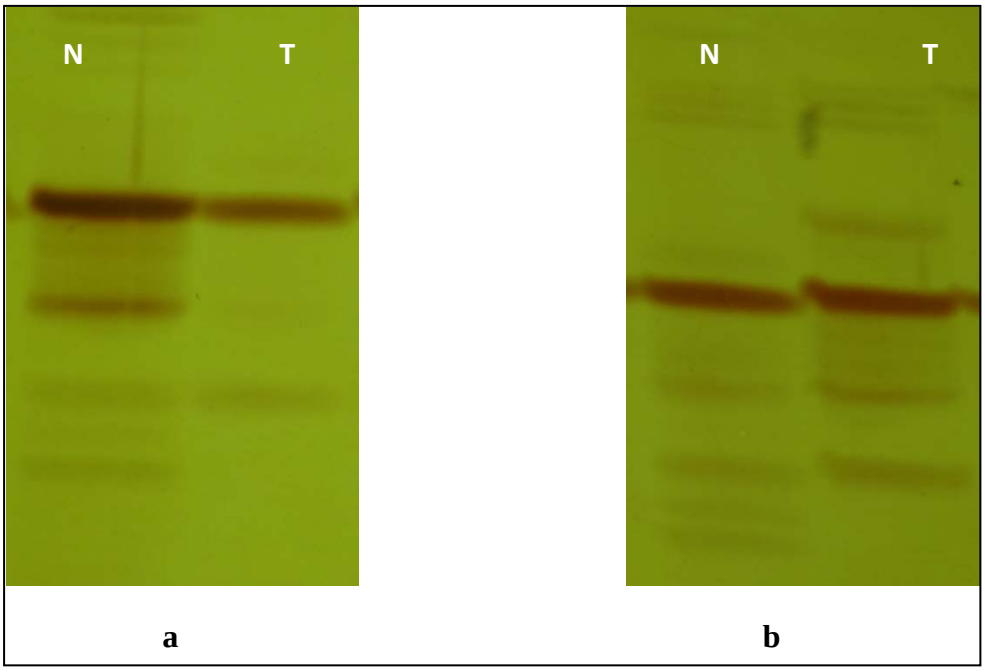


Figure 4.2 . (D21S1389) marker analysis in PAGE, gel shows samples (a) loss of heterozygosity LOH And (b) heterozygosity (RLOH).

The highest frequency of LOH and high polymorphic analysis, we get in this marker, On the other hand, the results of the comparative between the pathological characteristics (pathological etiology) and percentage of LOH %, ducks stage were 6(75%) ducks B, 1(12 %) ducks C, 1(12%) ducks D, the ratio between gender 5 (62.5%) females, 3(37.5%) males while the rate of age > 60 , ≤ 60 was equally 4(50%).

4.5.2 Results of Microsatellite Marker D21S1440

D21S1440 microsatellite marker at region 21q22.1 that has molecular weight 157-175 bp, we used polyacrylamide gel at concentration (11%), we get a clear results. The highest frequency of LOH we obtained at this marker 8 (19%), 27 (64.2%) RLOH, 3(7.1%) MSI, 4 (9.5%) NI from sum 42 CRC sample, see figure 4.3, and table 4.1

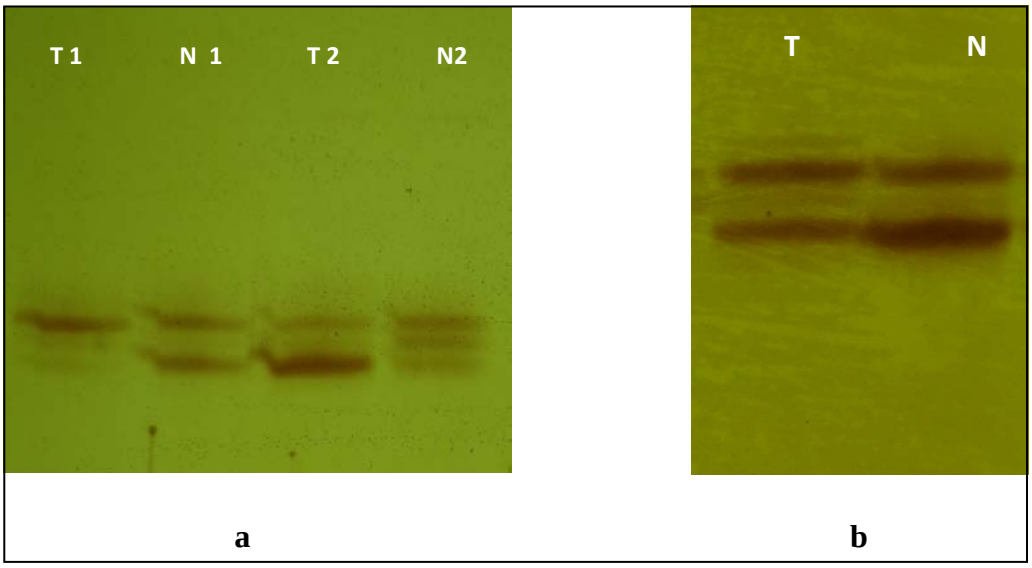


Figure 4.3. (D21S1440) marker analysis in PAGE, a (LOH), b (RLOH)

In figure 4.3. Marker D21S1440 in PAGE shows (a) loss of heterozygosity LOH in two designs (T1,N1) one band from 2 alleles disappeared in T1 (tumor sample) while in N2 (normal sample) had 3 alleles just 2 bend stay at T2, and in b heterozygosity (RLOH) clear at (T, N).

Furthermore, the results of the comparative the pathological characteristics and percentage of LOH %, ducks stage was 3(37 %) ducks B and 5(62 %) ducks C, the ratio between gender 5(62.5%) males, 3 (37.5%) females and the rate of age > 60, ≤ 60 it was equally 5 (50%).

4.5.3 Results of Microsatellite Marker D21S1981

D21S1981 microsatellite marker at region 21q22.3 which molecular weight 195 bp we used polyacrylamide gel at concentration (11%), we get a clear results, frequency of LOH we obtained at this marker 6(14.2%) LOH, 26(61.9 %) RLOH, 2(4.7%) MSI, 8(19%) NI, from sum 42 CRC sample, see figure 4.4, and table 4.1.

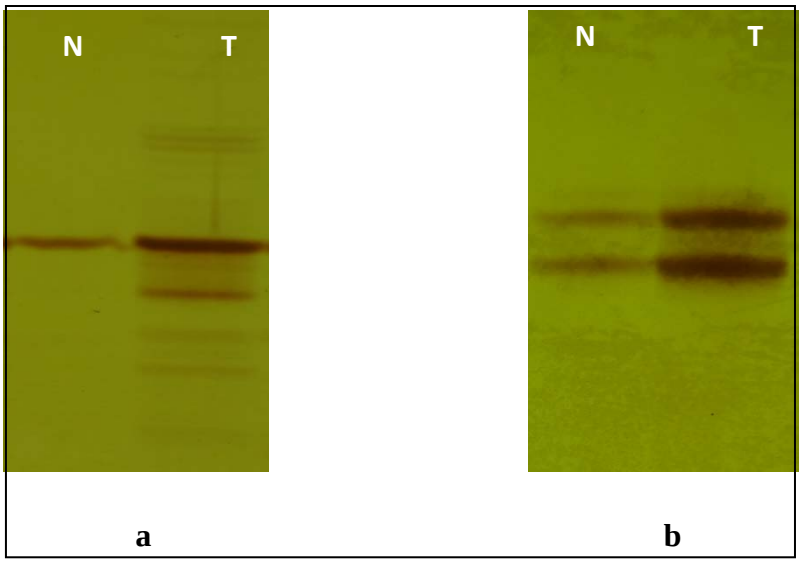


Figure 4.4. Marker D21S1981 analysis in PAGE

In above figures shows (a) loss of heterozygosity LOH and (b) heterozygosity (RLOH), As well , the results of the comparative between the pathological characteristics and percentage of LOH %, Ducks stage were 5(83%) ducks B, and 1(12%) ducks C, rate between gender 1(16.6%) males, 5(83.3%) females and the rate of age > 60, ≤ 60 it was1(16.6%), 5(83.3%) respectively.

4.5.4 Results of Microsatellite Marker D21S303

D21S303 microsatellite marker at region 21q22.12 which molecular weight 256 bp we used polyacrylamide gel at concentration (7%), we get a clear results. frequency of LOH we obtained at this marker about 4(9.5%) LOH, 27(64.2%) RLOH, 1(2.3%) MSI, 10(23.8%) NI, from sum 42 CRC sample, see figure 4.5, and table 4.1

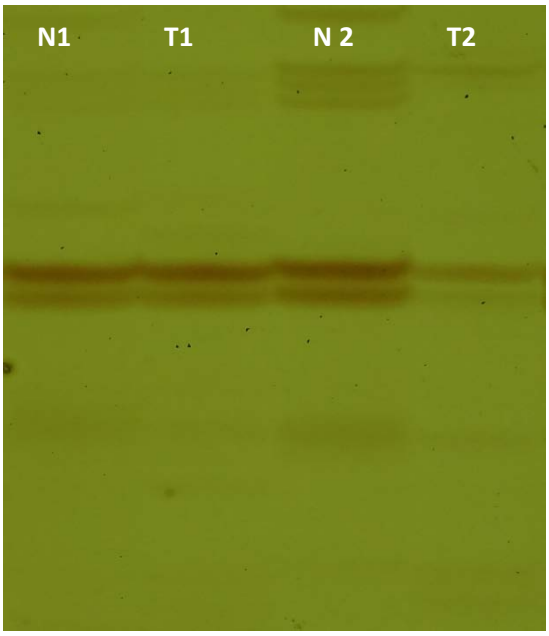


Figure 4.5. Marker (D21S303) analysis in PAGE , (N1.T1) RLOH and (N2,T2)LOH

Further , the results of the comparative between the pathological characteristics and percentage of LOH %, ducks stage were 1(25%) ducks B, 3(75%) ducks C, rate between gender 1(25%) males, 3(75%) females) and the rate of age > 60, ≤ 60 it was equally 2(50%).

4.5.5. Results of Microsatellite Marker D21S270

D21S270 microsatellite marker at regional 21q22.13 which molecular weight 199-223 bp, we used polyacrylamide gel at concentration (10%), we get a clear results. lower frequency of LOH, we obtained at this marker, 3(7.1 %) LOH, 28 (66.6%) RLOH, 2(4.7%) MSI, 9(21.4%) NI, from sum 42 CRC samples, see figure 4.6, and table 4.1

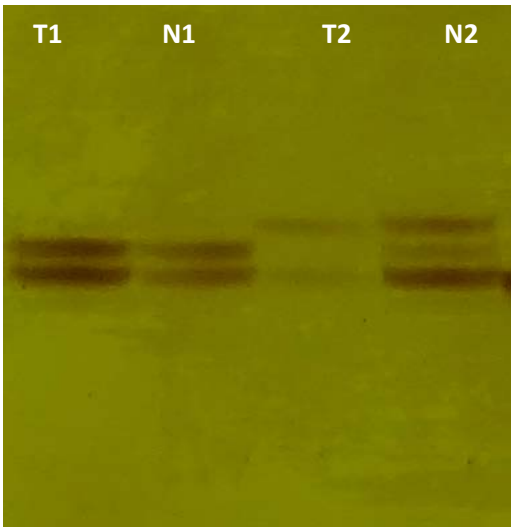


Figure 4.6. Marker (D21S270) analysis in PAGE. show (T1, N1) RLOH and (T2, N2) LOH.

In addition to, the results of the comparative between the pathological characteristics and percentage of LOH %, ducks stage were 1(33%) ducks A, 2(66%)ducks B, rate between gender 3(100%) were females and the rate of ages were ≤ 60 3(100%).

4.6. Numbers of Informative Cases Microsatellite Markers and LOH in all Cases

Detection of all microsatellite markers in sum 42 samples calculated. Informative polymorphic samples and LOH of each sample in markers recorded in a graphical, (See figure 4.6). Furthermore, microsatellite marker and percentage of analysis informative (RLOH with MSI), (LOH) were calculate with location each marker at chromosome 21 by a table 4.1.

Table 4.1. Informative cases of loss of heterozygosity in 42 cases.

Marker	location	No. of informative cases %	LOH+ %
D21S1839	21q11.2	42 (100)	8 (19)
D21S1440	21q22.1	38 (90.5)	8 (19)
D21S1981	21q22.3	34 (80.5)	6 (14.2)
D21S303	21q22.12	32 (76)	4 (9.5)
D21S270	21q22.13	33 (78.4)	3 (7.1)
Overall	**21q11-22**	**42**	**29 (57.1)**

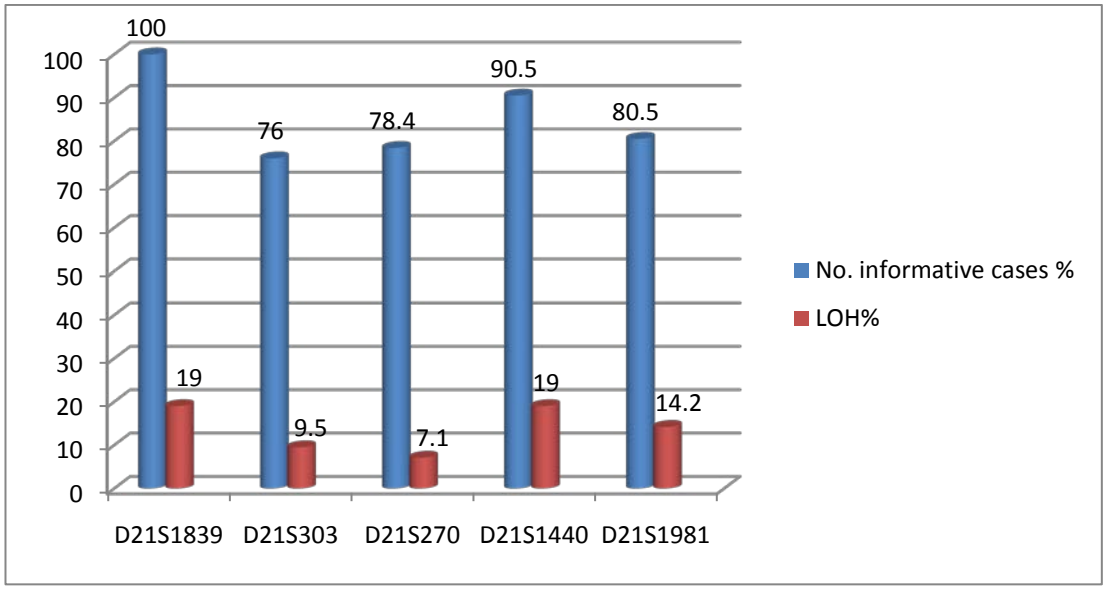


Figure 4.7. Figure show percentage of informative polymorphic samples, blue colure is RLOH, MSI and LOH) while the red color is just percentage of LOH.

4.7 Percentage Loss of Heterozygosity in 42cases at Microsatellite Markers

Examine 5 polymorphic microsatellite markers in a long arm of chromosome 21q11-22 region matched in 42 patients of CRC (tumor and normal) tissues identified frequency loss of heterozygosity of each locus of markers. We get 29(%57, 1) patients was detected LOH from sum 42 cases at least in one locus. The highest frequency of LOH was found on D21S1839 and in D21S1440 loci (%19) which we saw the same range, even in D21S1981 (%14.2), D21S303 (9%) loci the lowest frequency of LOH was on D21S270 (%7, 1) loci, see figure 4.8.

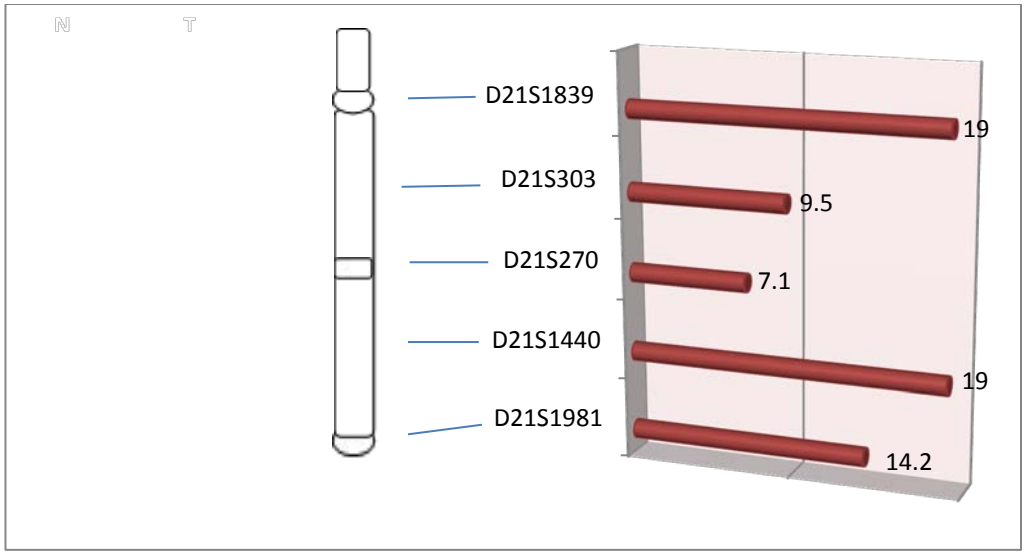


Figure 4.8. Percentage loss of heterozygosity in 42 cases at all microsatellite markers, chromosome 21 regions.

4.8. Relationship Between 21q11-22 Region and Clinic-Pathologic Factors

The relationship between clinicopathologic characteristics and frequency of LOH at 21q11-22 was analyzed using Fisher Exact Test. As follows age, ducks stage and gender. (We mentioned the ratios in analysis of markers). Frequency of LOH **21q11-22** had $p < 0.05$ (value 0.0001) considered extremely significant with gender, while had considered but not significant with ducks stage and is not statistically significant with age, (See table 4.2). It is concluded that LOH in region 21q11-22 is found to be associated with CRC in females, (odds ratio = 19.200).

Table 4.2 association between LOH and clinicopathologic characteristics of patients with CRC

Characteristic	**NO. of patients**	**LOH+**	**LOH-**	***P* value**	**Odds ratio**
Gender				0.0001	19.200
Male	42(21)	5	16		
Female	42(21)	18	3		
Age				0.1001	3.864
≤ 60	42(21)	17	4		
> 60	42(21)	11	10		
Ducks stage				0.3024	2.418
A&B	42(24)	19	5		
C&D	42(18)	11	7		

CHAPTER 5

DISCUSSIONS AND CONCLUSIONS

The rates CRC in developed countries are much lower in developing countries in Asia and Africa. In Iraq, a developing Asian country in Eastern Mediterranean region, these rates are around four folds less in Europe and North America. Nevertheless, CRC is still the seventh most common cancer among Iraqi population. Even the age rates are standardized ratio in almost the world (Al-allawi, et al., 2012).

Carcinogenesis multistep process leads to genetic alteration, tumor suppressor genes and oncogenes have role to be involved in most of this sequences, frequently in loss of the wild-type allele had seen at the appointed locus, so, loss of heterozygosity in tumor suppressor genes played a significant role in colorectal cancer transformation (Yamamoto, et al., 2011).

colorectal cancer and the interrelationship between tumor suppressor genes with progression disease were discussed by Ozaslan and aytekin in 2010. The results had get LOH of tumor suppressor genes,and that was observed at loci in different chromosomes (1p, 1q, 4q, 5q, 8p, 9q, 11q, 12p, 14q, 15q, 17p, 17q, 18p, 18q and 22q).

Loss of heterozygosity has been observed for sequence of loci of chromosome 21 in several solid tumors about (30-36) % that containing cancers of the head, neck, breast, pancreas, mouth, stomach, esophagus and lung. Furthermore, the reduced cases of solid tumors in individuals with Down syndrome (3 copies of q21) suggest that add dosage of some q21 genes keep them away such individuals from these tumors about (37±39 %) (Hattori, et al., 2000).

Silva et, al. reached to a results were indicating the presence of a tumor suppressor gene (RUNX1) in chromosome 21, the study was tested to (LOH) in seventeen with leukemia acute myeloid, six acute myeloid leukemia patients had shared a common region of LOH.

In this present study, we examined 5 polymorphic microsatellite markers in a long arm of chromosome 21q11-22 region which matched in 42 patients of CRC (tumor and normal) tissues identified frequency loss of heterozygosity of each locus of markers. We get 29 (%57, 1) patients was detected LOH from sum 42 cases at least in one locus, the highest frequency of LOH was found on D21S1839 (%19) loci, D21S1440 (%19) loci, D21S1981 (14.2 %) loci, D21S303 (9.5%) loci and the lowest frequency of LOH was on D21S270 (%7, 1) loci.

Sakata at el., were saw a detailed deletion map of chromosome 21 in gastric cancer after they analyzed 2 marker in region 21q22.1 . As well as Yamamoto et al., noted same deletion in a marker 21q11.2 region in squamous cell carcinoma, moreover in the same region on D21S1839 on loci 21q11.2, D21S1440 on loci 21q22.1 we detected LOH about 19%, 16% respectively. Deletion of alleles by loss of heterozygosity and imbalance chromosome may be associated with tumor suppressor genes. So, In oral cancer at 21q11.1 a high frequency of LOH was observed for D21S1410, D21S120, and D21S1433 had about 60% for each loci while, in our research, we used 21q11.2 region,frequency of LOH about 19% obtained, according to those results, region 21q11.1 may have a harbors to tumor suppressor genes in both oral cancer and in CRC. Reports of Navin et al., were based on analysis of single samples from whole tumors which the subpopulations were not separated by differences in region (6p22.1, 6p21.1, 17q21.32) had highest range of deletion while in loci (21q11) saw low ratio of deletion.

Park and his colleagues saw frequency of LOH at 21q22.3 region (34.2%) in patients of gastric cancer, as well as in our studies obtained highest frequency of LOH in region 21q22.3 about (16%). All these results indicates that locus might harbor to new tumor suppressor gene .adding , many studies supposed the trefoil factor family 1 (TFF1) located in this region, the possibility linked of one tumor suppressor genes at this region and involving carcinogenesis Recently, Hankey et al., indicated trefoil factor family (TFF) is missense mutations un clear yet, the TFF mutation seem rare in colorectal cancer while Serum of TFFI used to useful marker for patients with CRC, besides, Guleria and Sambyal studied at 56 patients of gastrointestinal GIT (most samples were colon cancer) their saw 9 cases loss at chromosome 21, Furthermore, the tumor suppressor genes RUNX1, ETS2, TFF1, TFF2, TFF3 and ERG implicated with cancers ,most of these genes localized on 21q22.2 and 21q22.3,

We identified frequmcy of LOH in 21q22.3 was about 14.2% ,it has been suggested there are some functions of TFF1, TFF2 and TFF3 genes are not a known exactly yet but there is a belief its maybe have role to protect the mucosa from insults to fixing the mucus layer, and affect healing of epithelium. Loss of these genes has been previously remembered in human GIT tumors and CRC, possibility that deletion were the same regions of those genes and tumorigenesis.

Several studies have investigated of the Runt-related transcription factors (RUNX), mitogen-activated protein kinas (MAPK1) and eukaryotic translation initiation factor 4E (eIF4E) are potentially involved in tumorigenesis. Studies genetic variation in RUNX 1, RNUX2, and RUNX3 with factor 4E (eIF4E) to how have the linked with (CRC). the statistically significant associations this genes with colon cancer had shown more than rectal cancer, analysis of a studies were reached about RUNX, MAPK1 and eIF4E and the association with TGF-b-signaling pathway given, the findings of interaction between genetic variants in genes under investigation with other genes in these pathway. The data emphasize the importance of this signaling pathway in the development of CRC with a CIMPþ phenotype and The TGF-b-signaling pathway has an important role in numbers conditions including colorectal cancer they believable that loss of TGF b growth control is a critical event in tumorigenesis (Slattery, et al., 2011).

Fijneman at el., reached through their studies, the RUNX1 is a novel tumor suppressor gene in the gastrointestinal tract. After conditional ablation of Runx1 expression in Apc, the analysis changed in to a high significant increase in the number of tumor cells, in all regions of the intestine.

The most important for guarantee survival in cancer cells is Loss of p53 activity which confers growth advantage by inhibiting apoptotic response required for tumor suppression. RUNX family, that composed of RUNX1, RUNX2, and RUNX3 are sequence-specific transcription factor, the function of these genes have big role in a many sides of cellular processes including development, differentiation, with tumorigenesis (Ito, 2014). Recently Ozaki at el., constructed role RUNX family. Even, they could describe a background of p53 and a functional collaboration between p53 and RUNX family in response to DNA damage.

Intriguingly, Several studies have investigated the RUNX1 play role in senescence-like growth arrest in primary murine fibroblasts, and this reply is lost in cell affect efficiency act p53. The results suggest investigating whether RUNX1 be involvement in p53-dependent apoptotic cell death followed by DNA damage. as is well known, p53 is tumor suppressor gene plays role at cellular stresses, following stimulate expression of downstream genes including chromosome 21 MDM2 and BAX, which regulate cell cycle and apoptosis, and angiogenesis loss of p53 is critical for tumorigenesis in many of cancers, Appears widely in colon cancer, approximately 40% to 50 % of colorectal carcinomas had deletion in p53 and related with aggressive tumors(Zhang, 2003).

in our study was investigating the relationship between LOH frequency and some of pathological etiology of the sampled tissues were performed by fisher's exact test, $P<0.05$ was considered as statistically significant, after using molecular techniques to detection, however no significant relationship was observed with tumorigenesis stages and ratio of ages. While the occurrence of LOH in region 21q11-22 was extremely significantly with gender (P=0.0001) so that associated with females,

Bottarelli at el., suggested frequency LOH in female CRCs was 46% with higher incidence in patients with tumor recurrence than in those whowere disease-free 0.01) and with a significant difference from adenomas ($P < 0.0001$) this analysis agreement with our studies frequency of LOH in **21q11-22** region had (p=0.0001) considered extremely significant,

,so as Wie at el were reported the role of gender in the development of colorectal cancer remains unclear. While Colorectal Cancer Facts & Figures Overall reported colorectal cancer incidence rates are about 35% to 40% higher in men than in women and same analysis had seen by Wang at 18qLOH had no significant association with gender and colorectal cancer cases.

siagel at el., in their studies saw rates of CRC was increasing among male and female under age 50 years, while the incidences in our analysis were 23 % under 50 years. Therefore must elucidate causes for this trend and identify potential prevention and early detection strategies need more research about risk factor affecting at CRC. Mastsuzaki at el., tried to investigate the prognostic value for LOH of chromosomes 4 and 14q in early-stage colorectal cancer (CRC), high level loss on each

chromosomes 4 and 14q, as an indicator prognostic in early-stage considered significant, while in our study tumorigenesis stages and relation with LOH was shown that were considered but no significant.

It is this study investigating the relationship between LOH and linkage with tumor suppressor genes like RUNX1, FTT1 in 21q11-22 region in colorectal cancer high frequency of LOH and extremely significant relationship was observed with gender and associated with females. Conclusion of this present study provides evidence of two minimal deletion regions, which may related with harbor putative tumor suppressor genes has been cause to progression and metastasis in sporadic colorectal carcinoma at 21q11-22 region.

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