



Colorectal Cancer and Folate pathway, 19-basepair deletion in particular: A review article

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Abstract

Background: Colorectal cancer is a major cause of mortality and morbidity in Jordan and worldwide. Abnormality of DNA methylation is a possible mechanism for the development of cancer. Dihydrofolate reductase (*DHFR*) is involved in DNA methylation. Genetic polymorphisms in the *MTHFR* gene may result in altered enzyme function, thus affecting cancer susceptibility and treatment response to antifolate cancer therapeutics.

Aim: The aim of this study is to review the 19-basepair deletion polymorphism of *DHFR* gene characteristics and CRC, chemotherapy and the relationship between them.

Results: According to studies, there was an association between cancer and genetics as well as different polymorphisms, particularly, *DHFR* gene and 19-19-basepair deletion polymorphism. The association was noticed in response and having cancer in life.

Conclusion: Cancer in general is one of the most important topics to be studied in present due to its high prevalence in the world. CRC by itself is one of the most vital issues in researches as it is highly variable and prevalent in different countries specially in Middle east and Jordan. The relationship between genetics, 19-basepair deletion particularly and different diseases are confirmed. However, its relationship with CRC is not very well defined. Accordingly, this topic needs to have multiple



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studies with different methodologies and in contrast to different factors, in order to have a clear statement about this issue.

Keywords: Colorectal cancer, DHFR gene, Jordan

1. Introduction

1.1 Cancer and colorectal cancer

Cancer is a term given to a group of related diseases. In all types of cancer, a group of cells start to divide irregularly and might propagate to other tissues (National Cancer Institute, 2015).

Cancer can start almost anywhere in the human body, which is made up of trillions of cells. In normal condition, human cells divide and multiply to make new ones as needed. When cells aged or become damaged, they die, and new cells take their place (National Cancer Institute, 2015). In cancer, however, this orderly process fails. As cells become more abnormal, aged or damaged cells survive at the time where they should be dead, and new cells form with no need or function. Tumors will be form which are abnormal growths as a result of irregular division of these extra cells. (National Cancer Institute, 2015).

Colorectal cancer (CRC) can be defined as a malignancy of the epithelial of the large bowel. The estimations say that 1 million new cases are being diagnosed yearly with a mortality rate of 52%. Most of the cases are diagnosed in the developed world with a rate of 64% men and 66% women which suggests that the environment as well as genetics is considered as factors that influence its incidence (Ferlay et al., 2010).

CRC is considered as the 3rd most common cancer and it causes death in the world taking the fourth record. The highest incidence of CRC has been recorded in the industrialized regions of North America, Central Europe, New Zealand and Australia and lower in Asia, Africa and South America (Ferlay et al., 2010).



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1.2. Genetics and colorectal cancer

At least one third of all CRC is thought to have a genetic component (Lichtenstein et al., 2000), but only about 5% are explained by the high-penetrance familial syndromes (Merg, Lynch, Lynch, & Howe, 2005). These syndromes, however, give an insight into some of the genes important in colorectal carcinogenesis.

The complexity of the role of folates in the cell arises from the involvement of the availability of one-carbon unit for nucleotide synthesis as well as for methylation of DNA, histones, and other proteins (Novakovic, Stempak, Sohn, & Kim, 2006). Folate levels alteration in the cell can be associated with irregular DNA repair and methylation, which include elevated global DNA hypo- and hypermethylation of promoters of tumor suppressor genes. Colon carcinogenesis involves all of these events (Ulrich, Curtin, Samowitz, et al., 2005). The key enzymes in folate pathway are methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), reduced folate carrier 1 (RFC1), and dihydrofolate reductase (DHFR). The activity of encoding these enzymes may be altered by polymorphisms in genes which might, consequently, influence colon carcinogenesis (Ulrich, Curtin, Potter, et al., 2005).

1.3. Importance of the study

CRC is considered as a major health issue in Jordan since it is ranked the first most common cancer among males and the second among females. It is also classified as the fourth most deadly type of cancer after lung, breast cancers and leukemia (Tarawneh & Nimri, 2012). Throughout the development of adenocarcinomas, a number of molecular, cellular, and histological changes cause shift from normal epithelium to adenoma and, finally, to cancer. These alterations can be caused both by genetic and non-genetic events (Blanco-Calvo, Concha, Figueroa, Garrido, & Valladares-Ayerbes, 2015).



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Folate metabolism is crucial for the DNA synthesis. At a cellular level, the blockage of the folate metabolism leads to a defective incorporation of uracil into the DNA chain. Recently, it is assumed that alteration of DNA methylation is associated with carcinogenesis and low methylation has been noticed in almost all cancers, especially colorectal cancer (Osian et al., 2009).

Dihydrofolate reductase (DHFR) is one of the main enzymes of the folate pathway catalyzes the NADPH dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) (Hayashi et al., 2012). This activity of DHFR enzyme is crucial for the biosynthesis of purine and thymidine, which is required for DNA polymerization (Selga, Noe, & Ciudad, 2008).

5-Fluorouracil (5-FU) was synthesized in 1956 as a nucleic acid metabolism inhibitor, and is one of the most widely used antineoplastic agents. The major growth-inhibitory effect of 5-FU has been associated with its metabolites, fluorodeoxyuridine monophosphate (FdUMP) and fluorouridine triphosphate (FUTP). Thymidylate synthetase (TS) is the target enzyme of 5-FU which F-dUMP binds covalently to it in the presence of N5, N10 methylenetetrahydro-folate (methylene THF), in order to block DNA synthesis by forming ternary complex. FUTP is incorporated into various class of RNAs and inhibits the normal metabolism of RNAs. The efficacy of cancer chemotherapy is greatly affected by the resistance to 5-FU. As a result it was urgent to study the mechanism of the cellular resistance to 5-FU. Many reports have said that TS increment is responsible for 5-FU resistance. Therefore, in 5-FU-resistant cells, DNA is formed though due to excess of TS which produce thymidine monophosphate (TMP). It has been found that a co-enzyme of TS called methylene THF had to increase in 5-FU resistant cells in order to produce TMP normally. This methylene THF is a reduced form of DHF, consequently, DHFR level would be high in 5-FU resistant cells so that enough amount of methylene THF would be supplied (Konishi et al., 1990).



1.4. Aim of the study and research questions

The aim of this study is to review the *19-bp deletion* polymorphism of DHFR gene characteristics and CRC, chemotherapy and the relationship between them.

Based on this our research questions are:

- What are the main factors that made CRC one of the most studied topics by researchers?
- What are the variables in CRC?
- What is the role of genetics in Cancer in general? And in CRC particularly?

2. Literature Review

2.1 Colorectal cancer overview

Colorectal cancer (CRC) is a cancer occurs in the epithelial tissues of either the colon, the mucosal colonic polyps, or the rectum. Rectum is defined as the distal 15 cm of bowel, measured from the anal verge. The colon is located from the terminal ileum and the ileocecal valve to the beginning of the rectum (Cappell, 2005).

It is believed that genetic and environmental factors enhance the development of CRC. The risk of CRC increases with age however it could occur in patients of all ages (Tarawneh & Nimri, 2012).

CRC arises from benign neoplasms and develops into adenocarcinoma through a gradual histological progression sequence, proceeding from either adenomas or hyperplastic polyps/serrated adenomas. Genetic alterations are associated with specific steps in this polyp-adenocarcinoma sequence and are known to drive the histological progression towards colon cancer (Butterworth, 2006).

Patients' survival is directly linked to the metastasis of primary colorectal tumors and accounts for 90% of patients' mortality. About half of the subjects with CRC can be cured by surgery and multimodal treatment, but therapy choices are restricted especially for metastasized patients. This is demonstrated by 5-year-survival rates of



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higher than 90% for early stage patients, 65% for patients with regional lymph node metastases, and less than 10% in patients with metastatic disease (Koelzer et al., 2015).

2.2 Incidence and epidemiology

In 2015, an expected 1,658,370 new cases of cancer will be diagnosed in the United States and 589,430 people will die from the disease (National Cancer Institute, 2015).

The number of new cases of cancer (cancer incidence) is 454.8 per 100,000 men and women per year (based on 2008-2012 cases) (National Cancer Institute, 2015).

The number of cancer deaths (cancer mortality) is 171.2 per 100,000 men and women per year (based on 2008-2012 deaths) (National Cancer Institute, 2015).

The age-standardized incidence rate (ASR) of CRC globally is 20.1 per 100,000 males and 14.6 per 100,000 females. The ASR is 40.0 in males and 26.6 in females in the developed parts of the world; in less developed areas, the rates are 10.2 and 7.7, respectively (Ferlay et al., 2010).

The highest ASRs in males are observed in Australia/New Zealand (48.2), followed by North America (44.4) and Western Europe (42.9). At the other end of the scale, the rates in South-Central Asia (4.7) and Central Africa (2.3) are lowest (Ferlay et al., 2010).

Cancer is one of the leading causes of morbidity and mortality in Jordan. Cardiovascular disease comes the first at the scale of mortality with a percentage of 35.9 while cancer is considered as the second cause of death (14.6%) (Ferlay et al., 2010)

The total number of registered new cancer cases in 2010 was 6820, with a percentage of 72.2 Jordanians (4921 cases compared to 4798 cases in 2009), a number of (4849) invasive cancers were registered while the rest of registered Jordanian cases (21) were in situ carcinoma (Tarawneh & Nimri, 2012).



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CRC is one of the top ten prevalent cancers among Jordanian cancer patients of both sex, it takes the second place with a percentage of 11.5. It is the first prevalent cancer among Jordanian males with a percentage of 14.2. On the other side it is the second prevalent cancer among Jordanian females with a percentage of 9. It has been noted that the vast majority of CRC patients among Jordanian are found in fifteens and above age category with a percentage of 71.2 of Jordanian CRC female patients and 75.3% of Jordanian CRC male patients (Tarawneh & Nimri, 2012).

The male-to-female incidence rate ratio (IRR) among Jordanian CRC patients is equal to 1.03 (Freedman, Edwards, Ries, & Young, 2006).

2.3 Risk factors for colorectal cancer

The incidence of sporadic colon cancer is believed to be affected by several factors such as lifestyle, environmental factors, diet, and acquired somatic mutations (Mundade, Imperiale, Prabhu, Loehrer, & Lu, 2014). About 70%-85% of CRC cases are sporadic and the patients do not have known inherited risk factors. Only 15–30% of CRCs may have a main genetic component which gives a share in the development of CRC in first- or second-degree relatives. Hereditary nonpolyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP) are of the main causes of heritable colorectal syndromes (Mundade et al., 2014).

2.3.1 Environmental factors

The environmental changes, driven by the economic transition are referred to be the main cause of the rise of CRC incidence rate in developing regions (Center, Jemal, & Ward, 2009). Age and environmental factors, such as obesity, dietary patterns, heavy alcohol consumption and smoking, are thought to influence CRC risk (Bishehsari, Mahdavinia, Vacca, Malekzadeh, & Mariani-Costantini, 2014). According to Surveillance, Epidemiology and End Results (SEER) Program database, the risk of developing colorectal cancer increases more than 14 times among people of 50 years



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of age and older compared to those younger than 50 years (Bhattacharya, Bhattacharya, Basu, Bera, & Halder, 2014).

The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) expert panel informed that high intakes of dairy products, dietary fibres, nuts, fish, fruits and vegetables decreases CRC risk while high smoking, body mass index (BMI) and waist circumference, red and processed meat intake and alcohol consumption are related to a higher CRC risk (Aleksandrova et al., 2014).

Ulcerative colitis (UC) and Crohn's disease (CD) are considered primary Inflammatory Bowel Disease (IBD). A dramatic rise in incidence rates of CRC has been seen in IBD patients (Goldacre, Wotton, Yeates, Seagroatt, & Jewell, 2008). The development of IBD-associated colonic cancer IBDACa/Dys is associated with several factors such as disease duration, the extent of IBD disease, age of onset, degree of inflammatory response, and a family history of colorectal cancer (Xu et al., 2015).

An increased CRC risk has been marked with diabetes in most, but not all. Although consistency issues had been seen in epidemiologic studies of the relationship of diabetes with the risk of colorectal cancer, most studies are compatible with a positive association (Larsson, Orsini, & Wolk, 2005).

Acromegaly is a rare condition in which an increased risk of CRC has been explained by higher circulating levels of insulin-like growth factor-1 (Rokkas, Pistiolas, Sechopoulos, Margantinis, & Koukoulis, 2008).

2.3.2 Genetic factors

2.3.2.1 Molecular Pathways

CRC is a complex disease that develops through the effects of multiple genetic mutations and epigenetic changes that include genes regulating cell growth and differentiation (Mundade et al., 2014). Recently it was reported that between 15–30%



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of CRCs may have a major hereditary component, given the occurrence of CRC in first- or second-degree relatives (Taylor, Burt, Williams, Haug, & Cannon-Albright, 2010). Most of the colorectal heritable syndromes are due to either familial adenomatous polyposis (FAP) or hereditary nonpolyposis colorectal cancer (HNPCC) (Fearon, 2011).

Familial adenomatous polyposis (FAP) is an inherited syndrome characterized by the development of multiple adenomas in the colorectum, a high risk of colorectal cancer (CRC), and the existence of extracolonic manifestations (Leoz, Carballal, Moreira, Ocana, & Balaguer, 2015).

Lynch syndrome is an autosomal-dominant condition characterized by a predisposition to several adult-onset cancers, most commonly CRC. Lynch syndrome is caused by mutations in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2) that can be identified through clinical genetic testing (Burton, Hovick, & Peterson, 2012).

2.3.2.2 Polymorphisms

The human genome is composed of over three billion bases of DNA encoding somewhere between 25,000 and 30,000 genes (Lander et al., 2001; Venter et al., 2001). Multiple forms of genetic variation are present in the human genome, but the most common form is the single nucleotide polymorphism (SNP). SNPs are DNA variants where a single nucleotide at a fixed position in the genome is substituted with another (Orr & Chanock, 2008). Traditionally, SNPs have been defined as sequence alterations that are present in the general population with a minor allele frequency (MAF) of >1% and they have been regarded to result in neutral or benign phenotypic alterations (Collins, Brooks, & Chakravarti, 1998). It has been estimated that there are in excess of 10 million common SNPs within the genome occurring, on average, every 300-1,000 base pairs (Orr & Chanock, 2008). Although vast majority of SNPs are shared between populations, many are specific to populations or continental



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grouping of populations that share a recent history. This subset of SNPs is likely to give rise to the observable phenotypic differences in and between populations, including disease susceptibility and outcome (Orr & Chanock, 2008). In this regard, SNPs can be used to measure admixture in populations and may be utilized to map genes that could account for the differences in disease incidence between populations (Patterson et al., 2004; Shriver et al., 2005).

It has been estimated that up to 50,000 to 200,000 SNPs may be biologically important (Chanock, 2001; Risch, 2000; Sachidanandam et al., 2001). SNPs have the potential to directly contribute disease pathogenesis, acting in a variety of ways depending on where they occur. SNPs located within genes can have serious consequences for the function or structural stability of a protein if they change its primary structure (Orr & Chanock, 2008). Exonic SNPs that result in amino acid substitutions are referred to as nonsynonymous SNPs (nsSNPs). These are the best-characterized class of genetic polymorphisms as they are subject to detection bias and their functional effects are usually easily assayable (Orr & Chanock, 2008).

The relative severity of an amino acid substitution can be predicted by evaluating the biochemical properties of the amino acid side chain in question. The significance of amino acid substitutions can be assessed using algorithms, such as the Sorting Tolerant From Intolerant (SIFT) and PolyPhen (Ng & Henikoff, 2006; Ramensky, Bork, & Sunyaev, 2002). Exonic SNPs that do not alter protein's primary structure are called synonymous and were thought to be functionally uninteresting. However, these SNPs can affect mRNA stability and alter splicing signals in genes (Capon et al., 2004; Chamary, Parmley, & Hurst, 2006). SNPs in introns, regulatory and gene-distant regions can also be functionally important by affecting gene regulation.

Promoter SNPs can directly affect transcription of a gene while intronic SNPs may affect splicing mechanism. Even SNPs that occur in apparent gene deserts have been



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associated with disease risk (Zanke et al., 2007). Methodologies for prediction of functional intronic or regulatory SNPs are in their infancy (Orr & Chanock, 2008).

Other classes of genetic variants include short tandem repeats (STRs) and variable number tandem repeats (VNTRs), collectively known as microsatellites. They are often extremely heterogeneous within a population (Orr & Chanock, 2008). Copy number variants (CNVs) are structural variants containing large regions of variable copy numbers and can have $MAF > 1\%$ (Iafrate et al., 2004).

It has been estimated that a pair of individuals from a population will differ by at least 11 CNVs (Sebat et al., 2004).

CNVs may encompass entire genes, promoter regions, and have dose effects (Iafrate et al., 2004). CNVs, therefore, may have an impact on phenotype, however the technology required detecting and assaying CNVs has not reached a level of accessibility and versatility as that of SNPs (Eichler, 2006).

Insertion-deletion variants (indels) occur when one or more base pairs (up to several kilobases) are present in some genomes, but not in others. An inversion variant is one in which the order of nucleotides gets reversed in a specific region of the chromosome. A well-known inversion variant has been identified on chromosome 17 in which ~900 kilobase interval is in the reverse order in approximately 20% of individuals with Northern European ancestry (Levy et al., 2007). Block substitutions, on the other hand, are strings of adjacent nucleotides that vary between genomes (Frazer, Murray, Schork, & Topol, 2009).

2.4 Folate metabolic pathway and DHFR

Methyl groups needed to synthesize nucleotides necessary for DNA biosynthesis and methylation of DNA, RNA, and proteins are supplied by cellular folates which function as donors and acceptors of methyl groups (Hubner & Houlston, 2009).



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DHFR is a ubiquitous enzyme seen in all organisms. The enzyme has a role in catalyzing the reduction to 5,6,7,8-tetrahydrofolate (THF) from 7,8-dihydrofolate (DHF) by stereospecific hydride transfer to the C6 atom of the pterin ring from the NADPH cofactor with concomitant protonation at N5 as it is shown in figure 1. DHFR has a core role in maintaining cellular pools of THF and its derivatives, which are fundamental for thymidylate and purine synthesis and accordingly for cell growth and proliferation. DHFR is the main source of THF, and as such is an vulnerability of rapidly proliferating cells: Several crucial anticancer and antimicrobial drugs mechanisms of action are based on targeting the enzyme. The seeking for the therapeutic target of the anticancer drug methotrexate (MTX) was part of the stimulant for more than 40 years ago for studies leading to the discovery of DHFR. MTX is considered one of the most potent chemotherapeutic agents in the treatment of lymphomas and leukemias . In the treatment of rheumatoid arthritis by MTX, DHFR is also the likely target. Trimethoprim is an important antibacterial agent which binds to bacterial DHFRs 105 times tighter than it does to vertebrate DHFRs. Pyrimethamine is the antimalarial agent which targets DHFR from *Plasmodium falciparum* (Schnell, Dyson, & Wright, 2004).



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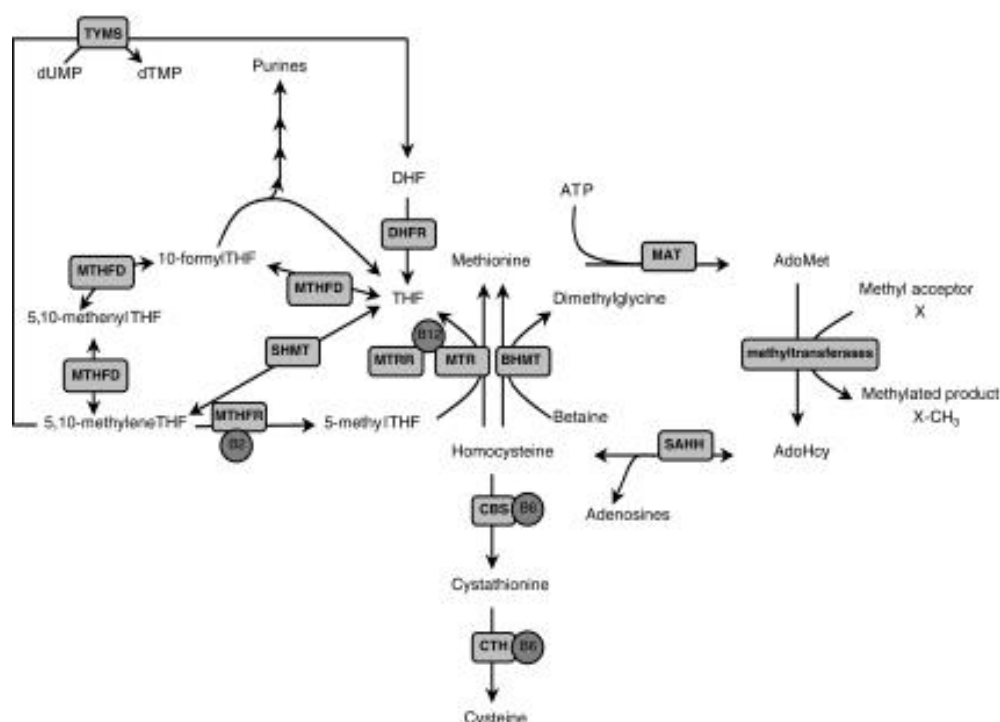


Figure (1): Schematic representation of the folate cycles and homocysteine metabolism (Blom & Smulders, 2011). *AdoHcy* S-adenosylhomocysteine, *AdoMet* S-adenosylmethionine, *AICAR* 5-aminoimidazole-4-carboxamide ribonucleotide, *SAHH* S-adenosylhomocysteine hydrolase, *ATP* adenosine triphosphate, *BHMT* betaine-homocysteine methyltransferase, *CBS* cystathionine β -synthase, *CTH* cystathionine γ -lyase, *DHF* dihydrofolate, *DHFR* dihydrofolate reductase, *dUMP* deoxyuridine monophosphate, *dTMP* deoxythymidine monophosphate, *FAICAR* formyl-AICAR, *MAT* methionine-adenosyltransferase, *MTHFD* methylenetetrahydrofolate dehydrogenase / methenyltetrahydrofolate cyclohydrolase / formyltetrahydrofolate synthetase, *MTHFR* methylenetetrahydrofolate reductase, *MTR* methionine synthase, *MTRR* methionine synthase reductase, *SHMT* serine hydroxymethyltransferase, *THF* tetrahydrofolate, *TYMS* thymidylate synthase



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2.5 The role of genetic polymorphism of *DHFR* 19-bp deletion in cancer

DHFR has a role in catalyzing the reduction of oxidized folates, like folic acid as well as DHF, to THF. Accordingly, folic acid supplementation benefits depend on DHFR activity. The reduction of DHF produced by TS to regenerate THF requires DHFR (Bailey, 2009).

The 19-bp deletion placed in intron 1 was reported on 2004. The current reference sequence of DHFR (NC_000005.8) contains the 19-bp deletion allele. The homozygosity frequency of the 19-bp deletion allele in whites is 17% to 22% (Bailey, 2009) .

Putative binding site is removed by the 19-bp deletion variant removes for the transcription factor Sp1 and accordingly DHFR expression might be affected. An increase in DHFR expression was observed by two studies due to the 19-bp deletion allele where no effect has been noticed by a third report (Bailey, 2009).

Studies on 19-bp deletion variant and Neural Tube Defects risk yielded conflicting results. In a large cohort, the combination of del/del genotype and multivitamine use was associated with 50% increase in breast cancer risk (Bailey, 2009).

3. Conclusion

Cancer in general is one of the most important topics to be studied in present due to its high prevalence in the world. CRC by itself is on of the most vital issues in researches as it is highly variable and prevalent in different countries specially in Middle east and Jordan. The relationship between genetics, 19-basepair deletion particularly and different diseases are confirmed. However, its relationship with CRC is not very well defined. Accordingly, this topic needs to have multiple studies with different methodologies and in contrast to different factors, in order to have a clear statement about this issue.



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