RELATION OF HOMOCYSTEINE AND FOLIC ACID IN CARCINOGENESIS- REVIEW WITH REVIEW OF LITERATURE

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***ABSTRACT*: Oral cancer is the commonest cancer in India, accounting for 50–70% of total cancer mortality and accounts for highest incidence among Asian countries. It affects anterior tongue, cheek, floor of mouth, gingiva or any other part of the oral cavity. The incidence of Oral Squamous Cell Carcinoma (OSCC) is high in several countries. Furthermore, the intraoral location differs in different population groups. These observations in turn provide a pointer towards the etiological agents involved. As the oral cavity is easily accessible for visual examination, oral cancer can be detected at an early stage. Nevertheless, in many tropical countries, in most instances patients with this disease seek medical attention only at an advanced stage, leading to poor prognosis and postoperative disfigurement. OSCC can be defined "as a malignant epithelial neoplasm exhibiting squamous differentiation and characterized by the formation of keratin and/or the presence of intercellular bridges". This is the most common malignant neoplasm of the oral cavity. Although it may occur at any intraoral site, certain sites are more frequently involved than others. The variation in the distribution of OSCC is due to the differences in clinical appearance, the nature of the lesion and particularly the prognosis.**

***KEYWORDS*: HOMOCYSTEINE, FOLIC ACID, ORAL CANCER, SQUAMOUS CELL CANCER, METHYLATION.**

INTRODUCTION

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy. Squamous cell carcinoma of the oral cavity represents over 90% of oral cancer onset. Early diagnosis plays a crucial role in the treatment and prognosis of the disease. Despite advances in all areas of diagnosis and treatment, the prognosis of patients with oral Squamous cell carcinoma has remained unchanged during the last two decades, and efforts toward early detection and prevention have not been entirely successful [1,2,3].

Molecular abnormalities in oral carcinogenesis have been extensively studied and mainly include genes involved in the control of the cell cycle, presumably resulting in a growth advantage in the altered cell population. Carcinogenesis is also associated with metabolic alterations, often nonspecific, might promote or derive from tumor progression. Thus, analysis of metabolic disorders may be a valuable approach to understanding the biochemistry of tumors and may provide a means to identify new targets for therapy [4].

Homocysteine is an amino acid with a free sulfhydryl group which does not occur in the natural human diet but is an essential part of the metabolism of methionine and it is produced as a result of methylation reactions.

It has recently been found that hyperhomocysteinemia is linked with cancer, which leads to the suggestion that homocysteine can also be regarded as a tumour marker. The main enzymes of homocysteine metabolism have vitamin cofactors like vitamin B6 or B12. This is why vitamins' level is often an important factor to be considered in homocysteine research and treatment even though vitamins cannot be usually treated as strict determinants of homocysteine. Since homocysteine levels are elevated in several diseases; a lot of research has been directed at methods of its measurement. Homocysteine exists in blood in three forms — protein-bound (about 80%), oxidized (homocysteine or homocysteine– cysteine, about 18%) or free (about 1%) [5].

Folic acid, one of the B vitamins, acts as cofactor in numerous biochemical reactions through its ability to donate or accept one-carbon units. Mammals are unable to synthesize folic acid de novo and so must either obtain it from the diet or from microbial breakdown in the gut. Folate deficiency has been reported to be the most common vitamin deficiency, affecting 10% of the general adult population and upto 60% of juveniles or the elderly in low socio-economic groups [6].

Folate plays an essential role in one carbon transfer involving the re-methylation of homocysteine to methionine, which is a precursor of S-adenosylmethionine, the primary methyl donor group for most biological methylations. DNA methylation is an important factor in gene expression, chromosomal modifications and aberrations. Currently, the most popular hypothesis is the promoter hypermethylation of key tumor suppressor genes[7].

DNA methylation is a regulator of gene transcription, and its role in carcinogenesis has been a topic of considerable interest in the last few years. Alterations in DNA methylation are common in a variety of tumors as well as in development. Of all epigenetic modifications, hypermethylation, which represses transcription of the promoter regions of tumor suppressor genes leading to gene silencing, has been most extensively studied. However, global hypomethylation has also been recognized as a cause of oncogenesis. New information concerning the mechanism of methylation and its control has led to the discovery of many regulatory proteins and enzymes. The contribution of dietary folate and methylene terahydrofolate reductase polymorphisms to methylation patterns in normal and cancer tissues is under intense investigation [7].

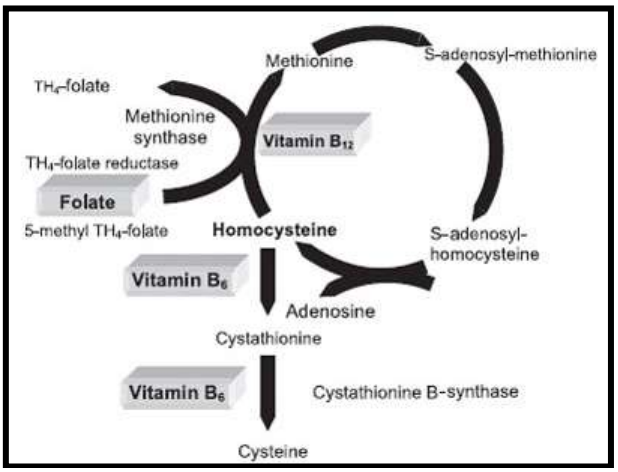
FOLATE- Pathogenesis and Mechanisms Involved.

Folic acid, also known generically as folate or folacin, is a member of the B-complex family of vitamins, and works in concert with vitamin B12. Folic acid functions primarily as a methyl-group donor involved in many important body processes, including DNA synthesis [8].

On a very simplistic level, cancer is thought to arise because of an excess of DNA damage and/or the inappropriate expression of critical genes. Folate has consequently been of particular interest as a potential cancer protective agent because of the important roles it plays in nucleotide synthesis, as well as in the biological methylation of molecules such as DNA, RNA, proteins, and the phospholipids [9].

Epidemiologic studies have observed that diminished folate status is associated with cancer of the cervix, colorectum, lung, esophagus, brain, pancreas, and breast. Among these, epidemiologic support for such a relationship is clearly most compelling for colorectal cancer [9]. Folate deficiency also has been considered as an important factor in alcohol-related enhancement of rectal carcinogenesis because alcohol alters normal folate metabolism. Although some animal studies support the epidemiologic concept that dietary folate is protective against selected cancers [10,11].Human intervention trials, designed to determine whether individuals at increased risk of cancer have that risk reduced by supraphysiologic doses of folate, have been performed almost exclusively in regard to cancer of the uterine cervix and colorectum[12,13].

Folate Metabolism:Figure 1



The sole biochemical function of all of the coenzymatic forms of folate in mammalian systems appears to be mediating the transfer of one-carbon units. Within the scope of this function is the synthesis of S-Adenosyl methionine (SAM) a methyl donor used widely for biological methylation reactions, and de novo deoxynucleoside triphosphate synthesis. Each of these two biosynthetic pathways is a means by which folate plays a major role in DNA metabolism. It is through disturbances in normal

DNA, and possibly RNA, metabolism that folate depletion appears to produce its pro-carcinogenic effects. Methionine is regenerated from homocysteine in a reaction catalyzed by 5-methyltetrahydrofolate (methylTHF): homocysteine methyltransferase: this is a reaction for which 5-methylTHF serves as both a cofactor and substrate [9].

An alternative mechanism for the regeneration of methionine which does not require folate also exists-the methylation of homocysteine by betaine-although the latter reaction seems to only be operative in the liver and kidney. Methionine, in turn, is converted to SAM in a reaction catalyzed by methionine adenosyl transferase. SAM then donates the labile methyl group it derived from 5-methylTHF for over 80 biological methylation reactions, including an array of reactions whereby specific sites within DNA and RNA become methylated. Although the alternative betaine pathway may partially compensate, dietary folate depletion alone is a sufficient perturbing force to diminish SAM pools [14]. The synthesis and turnover of deoxynucleoside triphosphate (dNTP) pools are tightly coupled to DNA synthesis. Since dNTPs are the immediate substrates for the polymerases involved in DNA replication and repair, the fidelity of DNA synthesis is critically dependent on the correct balance and availability of deoxynucleotides [9].

Folate-derived one–carbon groups are essential for the de novo synthesis of the pyrimidine, thymidylate, as well as the purines. In mammalian cells the de novo synthesis of thymidylate from deoxyuridylate is a rate-limiting step for DNA synthesis and requires 5, 10-methylene tetrahydrofolate as a coenzyme. When the dietary methyl supply is inadequate, such as in folate depletion, the use of folate coenzymes for biological methylation and nucleotide synthesis appear to compete. As SAM concentrations decrease, compensatory mechanisms increase the conversion of 5, 10-methyleneTHF to 5-methyl- THF, an irreversible reaction, and thereby compromise folate availability for de novo nucleotide synthesis [14].

Folate deficiency and associated Carcinogenesis:

Altered DNA methylation-

There is considerable evidence that aberrant DNA methylation plays an integral role in oncogenesis. First, a decreased level of genomic methylation is nearly universal finding in tumorigenesis: this has been observed in cancers of the colon, stomach, uterine cervix, prostate, thyroid and breast [9].

This decrease in genomic methylation appears early in carcinogenesis, and appears to precede the more well described mutation and deletion events that occur later in the evolution of cancer. Genomic hypomethylation has been observed in some animal models of carcinogenesis [15].

Gene-specific hypomethylation may occur even in the absence of genomic hypomethylation and is probably a more important event in carcinogenesis since the prevailing theories of carcinogenesis emphasize damage which occurs at critical loci within DNA. Site-specific aberrancies in DNA methylation within critical genes are observed in neoplastic tissues, and include both foci of hypomethylation and hypermethylation [16].

The induction of genomic hypomethylation in human lymphocytic DNA has been demonstrated in healthy human volunteers who were placed on a long-term folate deficient diet and this effect was reversible when the deficiency was corrected [17]. Supportive evidence is available from a recent observational study where serum folate levels as well as folate concentrations in the uterine cervix were significantly correlated with genomic DNA methylation in a study of cervical intraepithelial neoplasia [18].

Folate depletion has been shown to induce hypomethylation of the coding region of the p53 tumor suppressor gene even in the absence of genomic hypomethylation [19]. Conversely, supplemental folate has been shown to reverse the hypomethylation of this region which occurs in association with chemical carcinogenesis. This region within the p53 gene that is particularly susceptible to hypomethylation by folate depletion or chemical carcinogens is precisely that region that is most frequently mutated in human cancer [20].

Altered RNA methylation:

Like DNA, a wide variety of RNA species are methylated at specific sites by SAM mediated reactions. In some instances, the 59-methyl cap of RNA is methylated and in other instances, internal nucleotide residues are methylated. Although the precise functions of RNA methylation sites are only now becoming apparent, it appears that these patterns of methylation in RNA are also judiciously guarded by the cell and serve important purposes in maintaining stability of the RNA species and facilitating transport across the nuclear membrane [21].

De-methylation of tRNA was shown with a severe, methyl deficient diet [22]. Folate depletion alone is sufficient to demethylate some RNA species such as small nuclear RNA (snRNA) species which is a critical component of the machinery necessary for maturation of messenger RNA [23].

Disruption of DNA integrity*:*

Folate deficiency induces breaks in chromosomes and such breaks are associated with an increased risk of cancer in humans. More recently, folate deficient conditions in both cell culture and animal experiments have been shown to create an excess of breaks in the phosphodiester backbone of DNA, which is presumed to be the molecular basis for chromosomal breaks.There are several mechanisms by which folate deficiency might create such breaks: these include the incorporation of uracil from the cellular nucleotide pool into DNA and by in situ deamination of cytosine.

Folate deficiency reduces thymidylate synthesis from deoxyuridylate and the ensuing nucleotide imbalance increases the misincorporation of uracil bases into DNA as most DNA polymerases do not effectively distinguish between deoxyuridylate and thymidylate. Uracil in DNA is excised by a repair glycosylase, and in the process a transient single-strand break develops in the DNA.

Simultaneous removal and repair of two adjacent uracil residues on opposite strands can result in a double-strand DNA break, further exacerbating genetic instability. Unrepaired double-strand DNA breaks enhance cellular transformation in culture and increase cancer risk. Excessive DNA uracil content, as well as increased numbers of chromosomal breaks is observed in folate deficient humans, and both defects are reversed by folate administration.

Choi.S.W et al found that lymphocytic DNA from subjects with the MTHFR polymorphism is significantly less methylated than DNA from wild type subjects. These observations suggest that the protective effect of the polymorphism may be conveyed by an alteration in the forms of folate contained within the cell, and it explains how the protective effect might be operable even when total folate levels are normal [24].

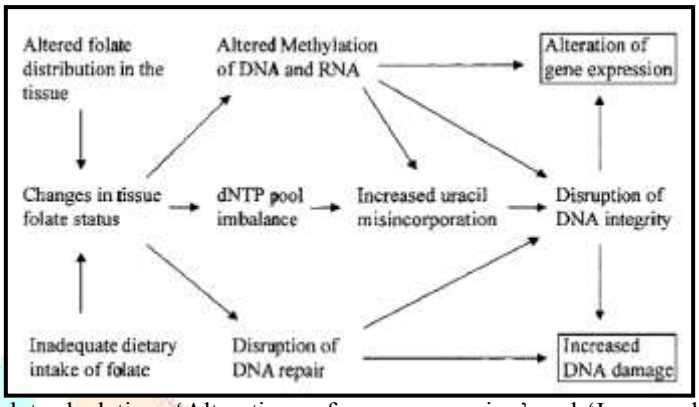


Figure2: Molecular effects of folate depletion. 'Alterations of gene expression' and 'Increased DNA damage' are enclosed in boxes to emphasize the concept that these are the two pathways through which carcinogenesis are enhanced [9].

Nancy Potischman et al suggested folate supplementation shows a significant improvement in cervical dysplasia [25].

Chandrika J Piyathilake et al conducted a cross sectional study among 39 smokers and 60 non recurrent smokers to evaluate the effect of cigarette smoking on folate and B-12 concentrations in the circulation and in tissues directly expose to cigarette smoke. Their study suggested that plasma and RBC concentrations of folate are considerably reduced shortly after smoking [26].

Susan J Duthi described two principal mechanisms through which low folate status may increase the risk of malignancy. Folate deficiency, by reducing intracellular S-adenosylmethionine , can alter cytosine methylation in DNA, leading to inappropriate activation of proto-oncogenes and induction of malignant transformation. Alternatively, folic acid is crucial for normal DNA synthesis and repair. Folate deficiency may cause an imbalance in DNA precursors, uracil misincorporation into DNA, and chromosome breakage [27].

Edward Giovannucci et al stated that folate deficiency enhances intestinal carcinogenesis in several animal models and the adverse effects of alcohol are accentuated when folate or methionine intake is low [28].

Young-In Kim et al suggested potential mechanisms of the folate deficiency-mediated colorectal carcinogenesis. 1) Aberrant genomic and site-specific DNA methylation 2) Abnormal apoptosis 3) MTHFR polymorphisms and related gene-nutrient interactions 4) DNA damage, uracil misincorporation, impaired DNA repair 5) Increased mutagenesis 6) Hyperproliferation [29].

Giovanni Almadori, MD et al observed that patients with head and neck squamous cell carcinoma or with laryngeal leukoplakia present a significant reduction in plasma folate levels. Their study assessed the effectiveness of folic acid as a chemopreventive agent in patients affected by glottis laryngeal leukoplakia [30].

Andrea Nacci MD et al reported metabolic alterations in homocysteine, folate, and vitamin B12 levels, especially hypofolatemia, could be associated with laryngeal cancer [31].

D. Aune et al found inverse associations between high intake of dietary folate and risk of cancers of the oral cavity and pharynx, esophagus, upper aerodigestive tract, colorectum and kidney and, in addition, with cancers of the larynx, stomach and breast [32].

HOMOCYSTEINE- Pathogenesis and Mechanisms Involved

Homocysteine (Hcy) is largely derived from cellular methionine, an essential amino acid drawn from dietary intake. Intracellular homocysteine is normally secreted extracellularly, at rapid rates. Consequently, the concentration of homocysteine in the plasma and urine reflects the balance between intracellular homocysteine production and utilization, while the blood concentration of homocysteine reflects the cellular homocysteine concentration.

In the circulating blood, the majority of the homocysteine binds to albumin, forming a disulfide linkage. Approximately 10% to 20% of the Hcy also exists as a mixed disulfide with cysteine or with homocysteine itself.

Very little Hcy is present in the circulating blood in a free reduced form (1%). Current procedures employed for the measurement of plasma homocysteine concentrations measure the total concentration of homocysteine (tHcy), which includes albumin bound homocysteine, homocysteine in all of the disulfide linkages, and the free reduced form of homocysteine.

According to Jacobsen the normal range of plasma tHcy for ''healthy adults'' is 5–15 µmol/l; 15 to 25 µmol/l indicates mild hyperhomocysteinemia; 25 to 50 µmol/l is considered intermediate hyperhomocysteinemia and > 50 µmol/l, severe. It was recommended that plasma tHcy be kept under 10 µmol/l [33].

Homocysteine Metabolism:

There are three major metabolic reactions involving homocysteine that is remethylation, transsulfuration and transmethylation. The remethylation reaction involving the conversion of Hcy to methionine by the addition of a methyl group to homocysteine is probably the most important reaction affecting plasma tHcy concentration. With the remethylation reaction, the enzyme methionine synthase is the major enzyme catalyzing the conversion of homocysteine to methionine by adding a methyl group to the homocysteine molecule. The transsulfuration reaction does not occur frequently. The transmethylation reaction appears to have the least impact by far on the plasma tHcy concentration. The enzyme methionine synthase requires 5-methyltetrahydrofolate (5-methyl-THF) as a methyl donor. Only in the liver, the conversion of homocysteine to methionine is catalyzed by betaine-homocysteine methyl transferase. In fact, additional enzymes are required for the conversion of THF back to 5-methyl-THF, forming a cycle for replenishing 5-methyl-THF. The most important of these enzymes is 5, 10- methylene-THF-reductase, which converts 5, 10-methylene THF to 5-methyl-THF. This reaction requires B12. The enzyme 5, 10-methylene-THF-reductase is heat labile and can be subjected to mutation. Consequently, the enzyme activity is impacted by both genetic and environmental effects [34].

Any defect occurring with this enzyme, either due to inherited gene mutations or environmental effects, would impact the conversion of homocysteine to methionine and produce hyperhomocysteinemia. Since the folate (in the form of 5,10-methylene tetrahydrofolate, a predominant cellular form) serves as the substrate and B12 the cofactor for this reaction, any deficiency in folate or in B12 will also slow the reaction rate and eventually lead to elevated plasma homocysteine.

Homocysteine as a tumor marker:

Elevated tHcy could be an early marker of carcinogenesis and also a sensitive marker for detecting recurrence. Serum tumor markers have been used most frequently for monitoring cancer patients during therapy [35]. Hyperhomocysteinemia caused by the proliferation of tumor cells can also be demonstrated from the study. In other words, monitoring tHcy reflects more accurately the tumor cell activity, making tHcy a better tumor marker. Several biochemical changes recently identified in association with hyperhomocysteinemia indicate that elevated tHcy in blood circulation creates a risk for cancer, and it is likely that hyperhomocysteinemia is a risk factor for carcinogenesis [36].

Folate deficiency

Hyperhomocysteinemia is frequently associated with folate deficiency. The depletion of folate is also known to interfere with the conversion of deoxyuridylate to thymidylate and leads to massive incorporation of uracil into human DNA, causing chromosome breaks, another reason why a folate deficiency would contribute to the increased risk of cancer . Elevated plasma tHcy has been found in hyper proliferative states such as psoriasis and many malignant diseases[70]. Folic acid not only modulates DNA repair, but also affects DNA strand breakage, decreases DNA stability in cells [37].

Oxidative stress

A major cause of endothelial injury and DNA damage may also be caused by the overproduction of oxygen free radicals generated from the oxidation of homocysteine. As reduced free homocysteine contains a free sulfhydryl group, free radicals including hydrogen peroxide can be generated upon oxidation of homocysteine, forming a disulfide linkage with free sulfhydryl group of albumin, cysteine or homocysteine. Apparently, it is the plasma level of reduced free homocysteine that affects and enhances oxidative stress. The endogenous attack on DNA by hydrogen peroxide and oxygen free radicals may generate many DNA adducts that can be detected in human cells. There are over 100 oxidative lesions in mammalian DNA, of which 8-hydroxyguanine is one of the most abundant.

Oxidation of DNA may cause gene mutation such as p53 and RAS gene, and eventually lead to carcinogenesis. Studies have also shown that oxidative DNA damage such as 8-hydroxyguanine accumulates in cancerous tissue.

Aberrant DNA methylation:

Recent work indicates that DNA methylation is an important player in both DNA repair and genome stability. Hypomethylation due to a reduced concentration of SAM and SAH has been reported in children with acute lymphoblastic leukemia and leukoencephalopathy treated with methotrexate. The effect of folate on DNA methylation has also been demonstrated by Piyathilake et al in Squamous cell carcinoma as well as in tissues at risk of developing SCC through aberrant methylation of DNA, another consequence of hyperhomocysteinemia.

It has been suggested that the inactivation of DNA repair pathways, which leads to an increased mutation rate and chromosomal instability, can initiate and accelerate the neoplastic process.

Homocysteine thiolactone

Increased concentrations of homocysteine thiolactone can be found in patients with hyperhomocysteinemia. The thiolactone can react with the primary amine of protein lysine residues under physiological conditions, a case of acylation. Malignant cells usually are unable to metabolize homocysteine thiolactone to sulfate as normal cells do. Therefore, homocysteine thiolactone accumulates in malignant cells, causing damage of cellular macromolecules. The oncogenic transformation of human cells has been found in association with the enhanced synthesis of Hcy thiolactone [38].

tHcy in blood circulation appears to be a better tumor marker for monitoring cancer patients during therapy because it not only accurately reflects the proliferation rates of tumor cells but also responds to tumor cell death [39].

Plasma homocysteine (Hcy), a well-known independent risk factor for coronary heart disease, is also a risk factor for cancer. Conceivably, tHcy may be used as a more accurate tumor marker for monitoring cancer patients during treatment, and

hyperhomocysteinemia as a risk factor for carcinogenesis. It also has the potential to be an early marker for carcinogenesis and a sensitive marker for the detection of recurrence [39].

The association of hyperhomocysteinemia with folate deficiency, oxidative stress, aberrant DNA methylation and Hcy thiolactone also provides strong support for viewing hyperhomocysteinemia as a risk factor for cancer [39].

Helga Refsum et al studied plasma homocysteine in 12 children with lymphoblastic leukemia. They evaluated alteration in plasma homocysteine with Acute Lymphoblastic leukemia prior to chemotherapy, during therapy, study showed decrease in the homocysteine level after therapy [40].

Shinji Oikawa et al indicated that the metal-dependent DNA damage through H_2O_2 is likely to be a more relevant mechanism for homocysteine carcinogenicity. To clarify whether hcy has potential carcinogenicity, they investigated formation of 8-oxodG, which is known to be correlated with the incidence of cancer, induced by homocysteine in human cultured cell lines [41].

Nadja Plazar and Mihaela Jurdana reviewed that increased homocysteine plasma concentration is related to a higher risk of coronary heart disease, stroke, peripheral vascular disease and malignancies [42].

Yun Jeong Lim et al found high level of plasma homocysteine, biochemical marker of folate status, is known to be a risk factor for cancer [43].

D. Aleksic et al identified increased homocysteine concentrations in children with malignant diseases compared with those with benign tumors confirming the possibility that the plasma concentrations of homocysteine are associated with defective metabolism of homocysteine in tumor cells and with the level of tumor progression [44].

Francesco Nicola Bartu et al suggested the role of Hcy and folates appears to be important in carcinogenesis of squamous cell carcinoma of the oral cavity [45].

Daniela Filippini Ierardi et al observed a significant difference in pre and post-treatment levels of Hcy in advanced breast tumors. Their research suggested that Hcy might be used as a prognostic biomarker for breast cancer [46].

Anna eleftheriadou et al indicated a positive correlation between hyperhomocysteinemia and hypofolatemia and the presence of Squamous cell carcinoma of head and neck suggesting folate as a dietary supplement might play a role in chemoprevention and the posttreatment care of squamouscell carcinoma of head and neck patients [47].

Forastiere A et al , Cravo ML et al, conformed Elevated homocysteine levels as an independent risk factor for colorectal cancer [10,48].Skiobola C et al , Refsum H et al found increased Hcy levels in hematological cancer [40,49].Pelucci C et al , Almadori et al investigated the folate and homocysteine status in SCCHN patients [4,50].Corona G et al, Mason JB et al, Schulz WA et al reviewed one-carbon metabolism factors have been associated with the risk of several malignancies [51,52,53].

James SJ et al ,Pelucci C et al , Almadori et al ,Parise O et al reported a significant inverse association between folate deficiency and squamous cell carcinoma of head and neck. Michael F.Fenech et al stated that deficient levels of folic acid and vitamin B12 are associated with elevated chromosome damage rate and high concentrations of homocysteine in the blood.

I Kato et al studied the relationship of baseline levels of serum folate and Hcy to the subsequent risk of colorectal cancer in a nested case-control study including 105 cases and 523 matched controls. The results of this study support the hypothesis that folate may be protective against colorectal cancer.

Paul F.Jacques et al suggested that folic acid fortification has had a substantial effect on plasma folate and homocysteine concentrations in a population based sample of middle aged and older adults [54].

Giovanni Almadori et al stated differences in serum levels of folate and homocysteine might arise from tumor development and consequent metabolic alterations or might precede and promote tumor progression. If hypofolatemia is a risk factor for head and neck carcinogenesis, it might suggest a role for folate as a novel chemopreventive agent both in patients with precancerous lesions and in patients with treated HNSCC at risk for loco-regional recurrence and second primary tumors [50].

Giovanni Almadori et al evaluated serum levels of folate, homocysteine, and vitamin B12 in patients with head and neck squamous cell carcinoma and in patients with laryngeal leukoplakia, a well known preneoplastic lesion suggesting role for folate deficiency as a risk factor in head and neck carcinogenesis.

Kuan-Ju Chen MS et al indicated that folate, vitamin B6 and B12 are independent nutritional factors associated with hyperhomocysteinemia [55].

Yesim Ozkan et al conducted a study in lung cancer patients using Fluorometric HPLC methods were used for the determination of Hcy. Increase t-Hcy, decreased folate levels were observed suggesting Hcy as a marker for cancer [56].

Andrea Nacci et al reported metabolic alterations in homocysteine, folate, and vitamin B12 levels, especially hypofolatemia, could be associated with laryngeal cancer [31].

Jennifer Lin et al suggested women with higher Hcy levels may be at an increased risk for breast cancer when their folate levels are low [57].

CONCLUSION

According to this review, it is understood that increased serum homocysteine levels and decreased serum folate levels can lead to Oral Squamous cell Cancer (OSCC). The present review concludes by finding an interesting association with serum homocysteine and folate levels in OSCC which could be useful as a biochemical "Tumor marker" and thereby providing insights into the onset and progression of the disease. Hypofolatemia which is a risk factor for oral carcinogenesis might

implicate folate as a novel chemo preventive agent, both in patients with potentially malignant disorders and in patients with treated OSCC at risk for loco-regional recurrence and second primary tumors.

REFERENCES

[1] Papadimitrakopoulou VA. Carcinogenesis of head and neck cancer and the role of chemoprevention in its reversal. Curr Opin Oncol 2000;12:240-5.
[2] Khuri FR, Lippman SM, Spitz MR, Lotan R, Hong WK. Molecular epidemiology and retinoid chemoprevention of head and neck cancer. J Natl Cancer Inst 1997;89:199-211.
[3] Armstrong W, Meyskens FL. Chemoprevention of head and neck cancer Otolaryngol Head Neck Surg 2000;122:728-35.
[4] Almadori G, Bussu F, Galli J, Cadoni G, Zappacosta B, Persichilli S, Minucci A and Giardina B: Serum folate and homocysteine levels in head and neck squamous cell carcinoma. Cancer *94(4)*: 1006-1011, 2002.
[5] Jacobsen D. Homocysteine and vitamins in cardiovascular disease.Clin Chem 1998;44:1833– 43.
[6] Susan J Duthie: Folic acid deficiency and cancer: mechanisms of DNA instability.Br Med Bull (1999) 55 (3): 578-592.
[7] Scott JM and Weir DG: Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovasc Risk *5*: 223-227, 1998.
[8] Choi SW and Mason JB: Folate and carcinogenesis: an intergrated scheme. J Nutr *130*: 129-132, 2000.
[9] Kim YI: Folate and carcinogenesis: evidence, mechanisms, and impications. J Nutr Biochem *10*: 66-88, 1999.
[10] Cravo ML, Mason JB and Dayal Y: Folate deficiency enhances the development of colonic neoplasia in dimethyl-hydrazinetreated rats. Cancer Res *52*: 5002-5006, 1992.
[11] Kim YI, Salomon RN and Graeme-Cook F: Dietary folate protects against the development of macroscopic colonic neoplasia in a dose responsive manner in rats. Gut *39*: 732-740, 1996.
[12] Butterworth Jr, C.E., Hatch, K.D., Gore, H., Mueller, H. & Krumdieck, C.L. (1982) Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. Am. J. Clin. Nutr. 35: 73–82.
[13] Butterworth Jr, C.E., Hatch, K.D., Soong, S-J., Cole, P., Tamura, T., Sauberlich, H.E., Borst, M., Macaluso, M. & Baker, V. (1992) Oral folic acid supplementation for cervical dysplasia: a clinical intervention trial. Am. J. Obstet. Gynecol. 166: 803–809.
[14] Miller, J.W., Nadeau, M.R., Smith, J., Smith, D. & Selhub, J. (1994) Folate deficiency induced homocysteinemia in rats: disruption of S-adenosylmethionine coordinate regulation of homocysteine metabolism. Biochem. J. 298: 415–419.
[15] Wainfan, E., Dizik, M., Stender, M. & Christman, J.K. (1989) Rapid appearance of hypermethylated DNA in livers of rats fed cancer promoting,methyl-deficient diets. Cancer Res. 49: 4094–4097.
[16] Hiltunen, M.O., Alhonen, L., Koistinaho, J., Myo¨ ha¨ nen, S., Pa¨ a¨ kko¨ nen, M., Marin, S., Kosma, V.M. & Ja¨ nne, J. (1997) Hypermethylation of the APC (adenomatous polyposis coli) gene promoter region in human colorectal carcinoma. Int. J. Cancer 70: 644–648.
[17] Jacob, R.A., Gretz, D.M., Taylor, P.C., James, S.J., Pogribny, I.P., Miller, B.J., Henning, S.M. & Sendseid, M.E. (1998) Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J. Nutr. 128: 1204–1212.
[18] Fowler, B.M., Giuliano, A.R., Piyathilake, C., Nour, M. & Hatch, K. (1998) Hypomethylation in cervical tissue: Is there a correlation with folate status? Cancer Epidemiol. Bio. Prevent 7: 901–906.
[19] Kim, Y.I., Pogribny, I.P., Basnakian, A.G., Miller, J.W., Selhub, J., James, S J. & Mason, J.B. (1997) Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am. J. Clin. Nutr. 65: 46–52.
[20] Kim, Y.I., Pogribny, I.P., Salomon, R.N., Choi, S.W., Smith, D.E., James,
[21] S.J. & Mason, J.B. (1996 b) Exon-specific DNA hypomethylation of the p53 gene of rat colon induced by dimethylhydrazine. Modulation by dietary folate. Am. J. Pathol. 149: 1129–1137.
[22] Camper, S.A., Albers, R.J., Coward, J.K. & Rottman, F.M. (1984) Effects of undermethylation on mRNA cytoplasmic appearance and half life. Mol. Cell. Biol. 4: 538–543.
[23] Wainfan, E., Moller, M.L., Maschio, F.A. & Balis, M.E. (1975) Ethionine- induced changes in rat liver transfer RNA methylation. Cancer Res. 35: 2830–2835.
[24] Choi, S.W., Lathrop Stern, L., Dzialo, H.M., Dolnikowski, G.G., Selhub, J. & Mason, JB. A common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene decreases genomic DNA methylation, but does not reduce DNA strand breaks, p53 methylation or uracil misincorporation: Implication for colorectal carcinogenesis. Gastroenterology 116: A303.1999
[25] Nancy Potischman, Louise A. Brinton, Virginia A. Laiming, William C. Reeves, Maria M. Brenes, Rolando Herrero, Francisco Tenorio, Rosa C. de Britton, and Eduardo Gaitan: A Case-Control Study of Serum Folate Levels and Invasive Cervical Cancer.cancer research 51, 4785-4789, September 15, 1999.
[26] Piyathilake CJ, Macaluso M, Hine RJ, Richards EW and Krumdieck CL: Local and systemic effects of cigarette smoking on folate and vitamin B-12. Am J Clin Nutr *60*: 559-566, 1994.
[27] Susan J Duthie: Folic acid deficiency and cancer: mechanisms of DNA instability.Br Med Bull (1999) 55 (3): 578-592.
[28] Edward Giovannucci: Epidemiologic Studies of Folate and Colorectal Neoplasia: a Review. J. Nutr. 132 : 2350S-2355S,2002
[29] Young-In Kim : Role of Folate in Colon Cancer Development and Progression. J. Nutr. 133: 3731S–3739S, 2003

[30] Almadori G, Bussu F, Navarra P, Galli J, Paludetti G, Giardina B, Maurizi M: Pilot phase IIA study for evaluation of the efficacy of folic acid in the treatment of laryngeal leucoplakia. Cancer 2006 Jul 15;107(2):328-36.
[31] Nacci A, Dallan I, Bruschini L: Plasma homocysteine, folate and vitamin B12 levels in patients with laryngeal cancer. Arch Otolaryngol Head Neck Surg 2008; 134: 1328 – 1333.
[32] D. Aune, H. Deneo-Pellegrini, A. L. Ronco, P. Boffetta, G. Acosta, M. Mendilaharsu & E. De Stefani: Dietary folate intake and the risk of 11 types of cancer: a case–control study in Uruguay. Annals of Oncology 22: 444–451, 2011.
[33] Jacobsen D. Homocysteine and vitamins in cardiovascular disease.Clin Chem 1998; 44:1833– 43.
[34] Fowkes FG, Lee AJ, Hau CM, Cooke A, Connor JM, Lowe GD. Methylene tetrahydrofolate reductase (MTHFR) and nitric oxide synthase (NOS) genes and risks of peripheral arterial disease and coronary heart disease: Edinburgh Artery Study.Atherosclerosis 2000;150:179–85.
[35] Wu J, Nakamura R. Human circulating tumor markers. Current concepts and clinical applications. Chicago: ASCP; 1997.
[36] Lily L. Wu, James T. Wu : Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clinica Chimica Acta 322 (2002) 21–28
[37] Duthie SJ, Narayanan S, Blum S, Pirie L, Brand GM. Folate deficiency in vitro induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal human colon epithelial cells. Nutr Cancer 2000;37:245–51.
[38] Jakubowski H, Goldman E. Synthesis of homocysteine thiolactone by methionyl-tRNA synthetase in cultured mammalian cells. FEBS Lett 1993;317:237–40.
[39] Lily L. Wu, James T. Wu : Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clinica Chimica Acta 322 (2002) 21–28
[40] Helga Refsum H, Wesenberg F, Ueland PM. Plasma homocysteine in children with acute lymphoblastic leukemia: changes during a chemotherapeutic regimen including methotrexate. Cancer Res 1991;51:828– 35.
[41] Shinji Oikawa, Katsuhiko Murakami and Shosuke Kawanishi: Oxidative damage to cellular and isolated DNA by homocysteine: implications for carcinogenesis. Oncogene (2003) 22, 3530–3538.
[42] 42)Nadja Plazar and Mihaela Jurdan: Hyperhomocysteinemia and the role of B vitamins in cancer Radiol Oncol 2010; 44(2): 79-85.
[43] Yun Jeong Lim, Jeong Hwan Kim, Sue K. Park, Hee Jung Son, Jae J. Kim, and Young-Ho Kim: Hyperhomocysteinemia is a risk factor for colorectal adenoma in women.j Clin Biochem Nutr. Sep 2012; 51(2): 132–135.
[44] D. Aleksic, D. Djokic, I. Golubicic, V. Jakovljevic, D. Djuric The importance of the blood levels of homocysteine, folic acid and vitamin B12 in children with malignant diseases. JBUON 2013; 18(4): 1019-1025.
[45] Fransesco Nicola Bartuli,Paolo Piva: Squamous cell carcinoma of the oral cavity and hyperhomocysteinemia.There'is a correlation?A preliminary study. International journal of clinical dentistry,volume6,Number3,219-225.
[46] Daniela Filippini Ierardi, Luis Gustavo Raimundo, Gimena Aguiar: Homocysteine as a Biomarker for Predicting Disease-Free Survival in Breast Cancer. Advances in Breast Cancer Research, 2013, 2, 24-31.
[47] Anna Eleftheriadou, Thomas Chalastras, Elisa Ferekidou: Association between Squamous Cell Carcinoma of the Head and Neck and Serum Folate and Homocysteine. Anticancer research *26*: 2345-23481.
[48] Forastiere A, Koch W, Trotti A and Sidransky D: Medical progress – head and neck cancer. N Engl J Med *345*: 1890-1900, 2001.
[49] Skibola C, Smith M, Kane E, Roman E, Rollinson S and Cartwright R: Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA *96*: 12810-12815, 1999.
[50] Almadori G, Bussu F, Galli J, Cadoni G, Zappacosta B, Persichilli S, Minucci A, Giardina B and Maurizi M: Serum levels of folate, homocysteine, and vitamin B12 in head and neck squamous cell carcinoma and laryngeal leukoplakia. Cancer *103(2)*: 284-292, 2005.
[51] Corona G, Toffoli G and Fabris M: Homocysteine accumulation in human ovarian carcinoma ascitic/cystic fluids possibly caused by metabolic alteration of the methionine cycle in ovarian carcinoma cells. Eur J Cancer *33*: 1284-1290, 1997.
[52] Mason JB and Levesque T: Folate effects on carcinogenesis and the potential for cancer chemoprevention. Oncology (Basel) *10*: 1727-1742, 1996.
[53] Schulz WA: DNA methylation in urological malignancies (Review). Int J Oncol *13*: 151-167, 1998.
[54] Paul F. Jacques, Sc.D., Jacob Selhub, Ph.D., Andrew G. Bostom, M.D., Peter W.F. Wilson, M.D., and Irwin H. Rosenberg, M.D. The Effect of Folic Acid Fortification on Plasma Folate and Total Homocysteine Concentrations.N Engl J Med 1999; 340:1449-1454.
[55] Kuan-Ju Chen MS, Wen-Harn Pan PhD, Feili-Lo Yang PhD, Ien-Lan Wei PhD, Ning-Sing Shaw PhD and Bi-Fong Lin PhD:Association of B vitamins status and homocysteine levels in elderly Taiwanese. Asia Pac J Clin Nutr 2005; 14 (3):250-255.
[56] Yesim Ozkan, Sevgi Yardim-Akaydin:Usefulness of Homocysteine as a Cancer Marker: Total Thiol Compounds and Folate Levels in Untreated Lung Cancer Patients. Anticancer Research *27*: 1185-1190 .
[57] Jennifer Lin,I-Min Lee,Yiqing et al:Plasma homocysteine and cysteine and Risk of breast cancer in women.Cancer Res 2010;70:2397-2405.