Epigenetic modulation and cancer: effect of metabolic syndrome?1–3

Sharon A Ross and John A Milner

ABSTRACT
The importance of epigenetics in the etiology of disease, including cancer development and progression, is increasingly being recognized. However, the relevance of epigenetics to the metabolic syndrome, and how it may affect cancer, is only beginning to capture the interest of the scientific community. This review focuses on data supporting the hypothesis that, in addition to the “thrifty genotype” and “thrifty phenotype” hypotheses, diet-induced changes in “epigenetic programming” during fetal and postnatal development may precipitate the metabolic syndrome. Thus, epigenetics may bridge both the thrifty genotype and thrifty phenotype hypotheses and provide a link between genes and the environment concerning disease predisposition to metabolic syndrome and its associated diseases. Am J Clin Nutr 2007;86(suppl):872S–7S.

KEY WORDS Epigenetics, cancer, nutrition, maternal diet, metabolic syndrome

THRIFTY GENOTYPE AND THRIFTY PHENOTYPE
The “thrifty genotype” and “thrifty phenotype” hypotheses have been proposed to explain the connections between early life environment and later development of adult disease, including those related to metabolic syndrome. The term thrifty genotype was coined by Neel in 1962 (1) to describe a theoretical concept about how survival occurred during historical periods of famine and how diseases of civilization develop during periods of feast and inactivity. The concept hypothesizes that to ensure survival during periods of famine, certain genes (thrifty genes) evolved to regulate efficient intake and utilization of fuel stores. According to the concept, diseases of civilization result from a discordance between certain features of our present-day environment (feast and inactivity) and our genetic make-up that evolved over time to fit the life of ancient humans.

The thrifty phenotype hypothesis was developed to explain observed associations between low birth weights, impaired glucose tolerance, and the metabolic syndrome (2). Epidemiologic observations suggested that poor growth in utero predisposes an individual to the later development of type 2 diabetes and hypertension in adulthood (3). In their hypothesis, Hales and Barker (4) described that low birth weight and long-term insulin resistance are adaptive for a developing organism if food supplies are scarce and likely to remain so over a sustained period. Thus, the thrifty phenotype is triggered by one or more signals of maternal malnutrition passing across the placenta to the developing fetus, essentially providing the fetus with a prediction of an impoverished nutritional environment into which it will be born. If the prediction proves wrong, however, and food supplies become abundant, the thrifty phenotype is maladaptive, becoming a risk factor for diabetes, obesity, and related disorders.

Both the thrifty genotype and the thrifty phenotype are in many ways plausible explanations to describe the apparent association between early life events and later disease outcome. However, it is becoming increasingly apparent that epigenetics may be a common path for the interaction of both genes and the environment. Changes occurring during early life may influence the predisposition to metabolic syndrome and subsequently several chronic diseases. Thus, the idea that epigenetic processes, such as chromatin remodeling and regulation of gene expression by DNA methylation, underlie developmental programming and interact with early nutritional exposures to influence disease in adulthood is gaining greater acceptance (5). This brief review provides a description of cancer epigenetic processes and highlights examples that suggest an epigenetic-diet interaction early in life may affect metabolic syndrome or chronic disease later in life.

CANCER EPIGENETICS
Epigenetics is the study of meiotically and mitotically heritable changes in gene expression that are not coded for by shifts in the bases within DNA (6). Epigenetic mechanisms have many layers of complexity, including DNA methylation, histone modifications, chromatin remodeling, and microRNAs as well as other noncoding regulatory RNA (7). It appears that the regulation or interaction of these layers affects whether the chromatin is open or closed or whether a gene is expressed or repressed. Modulation of epigenetic mechanisms enables, by definition, the alteration of cellular phenotype without altering the genotype; thus, epigenetic processes can be viewed as one possible mediator between genes and the environment, including diet, in expressing phenotype. An increasing number of diseases, including cancer and its development and progression, have been described as having a link to epigenetic dysregulation (7). The aberrant epigenetic landscape of the cancer cell has been characterized by genomic hypomethylation, CpG island promoter hypermethylation of tumor suppressor genes, an altered histone code for

1 From the Nutritional Science Research Group, Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.
3 Address reprint requests to SA Ross, National Cancer Institute, National Institutes of Health, 6130 Executive Boulevard, EPN 3157 MSC 7328, Bethesda, MD 20892-7328. E-mail: rosssha@mail.nih.gov.
critical genes, and a global loss of monomethylated and trimethylated histone H4 (7).

**EPIGENETIC MECHANISMS**

The regulation of epigenetic processes involves many strategies, including DNA methylation, histone modifications, chromatin remodeling, and noncoding regulatory RNA. In addition, a variety of regulatory proteins, including DNA methyltransferases, methyl-CpG binding proteins, histone-modifying enzymes, and chromatin remodeling factors, are involved in the overall epigenetic process. How these strategies and regulatory factors interact to regulate chromatin structure, dynamics, and gene expression is an active area of research, and recent understanding of these epigenetic mechanisms are highlighted below.

**DNA methylation**

DNA methylation is the covalent addition of a methyl group to 5th position of cytosine, which is largely confined to CpG dinucleotides. The distribution in the genome of the CpG dinucleotides at which DNA methylation occurs is asymmetric. In contrast with the relative paucity of CpGs in the genome as a whole, these dinucleotides can be clustered in small stretches of DNA termed CpG islands (8), which are found in the promoter regions of housekeeping, tumor suppressor, and some tissue-specific genes (9). Hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes is now firmly established as an important mechanism for aberrant gene inactivation (10). Other genomic methylation targets include imprinting regulatory elements, which are differentially methylated, and transposable elements, which are typically methylated (11). Thus, various physiologic processes are controlled by specific DNA methylation patterns, including genomic imprinting, inactivation of the X chromosome, regulation of tissue-specific gene expression, and repression of transposons (12).

DNA methylation is accomplished through the activity of specific enzymes, the DNA methyltransferases (DNMTs), which transfer a methyl group, in coordination with the universal methyl donor S-adenosylmethionine (SAM), to the cytosine of CpG dinucleotides. The better characterized DNMTs are DNMT3A, DNMT3B, and DNMT1 (11). DNMT3A and DNMT3B are mainly devoted to de novo methylation, whereas DNMT1, which has preferential activity for hemimethylated DNA, acts mainly as a maintenance methyltransferase (11).

Interestingly, there appear to be periods of temporal susceptibility to epigenetic modulation. Mammalian development, for example, is well characterized by bimodal DNA methylation reprogramming that occurs initially during germ cell development and then during preimplantation (13). Investigators have also suggested that additional windows of susceptibility exist during fetal and postnatal periods (14), and perhaps in puberty. More research is needed to examine DNA methylation modulation potential during these temporal and developmental windows. Moreover, both increases and decreases in DNA methylation have been associated with the aging process and may be involved in several age-related diseases (15). For example, methylation changes that occur in an age-related manner may include the inactivation of cancer-related genes. In some tissues, levels of methylated cytosines decrease in aging cells, and this demethylation may promote chromosomal instability and rearrangements, which increase the risk of cancer. In other tissues, such as the intestinal crypts, increased global hypermethylation may be the predisposing event that accounts for the increased risk of colon cancer with advancing age. The causes and mechanisms underlying age-related methylation changes are largely unknown and thus require more probing study.

**Histone modifications**

The fundamental repeating unit of chromatin is the nucleosome, which is composed of an octamer of 4 core histones (2 copies of the H2A, H2B, H3, and H4 histones) surrounded by 147 base pairs of DNA (16). Many covalent modifications have been discovered to occur at the amino acids that constitute the N-terminal tails of histones. Alone or in combination, these histone modifications are thought to be indispensable for the regulation of the continued repression, or expression, of genes. Thus, histone modifications of nucleosomes distinguish euchromatic (open) from heterochromatic (closed) chromatin states. In particular, acetylation of specific residues in histones H3 and H4 has been associated with an open chromatin configuration and a permissive gene transcription state (17). In addition to histone acetylation, the methylation, phosphorylation, ubiquitination, sumoylation, and biotinylation of histones has been shown (16). Histone acetylation is regulated by several enzymatic activities with the capacity to either transfer acetyl groups or induce histone deacetylation, which is associated with gene silencing. In addition to the loss of monocetylation and trimethylation of histone H4 as a common hallmark of human tumor cells, an imbalance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities also exists in cancer cells (18). The development of HDAC inhibitors for cancer chemotherapy is a current research emphasis (18).

**Chromatin remodeling**

DNA methylation and histone modification occur in the context of a higher-order chromatin structure. Chromatin remodeling plays a key role in the regulation of gene expression and is strongly influenced, in part, by posttranslational modifications of histones, including histone acetylation. Moreover, recent discoveries have characterized nonhistone proteins that modulate DNA-histone interactions and affect chromatin remodeling. For example, multisubunit complexes of the SWI/SNF proteins use the energy of ATP to mobilize nucleosomes and allow access of the transcriptional machinery (19), and massive repressive complexes, such as the polycomb group gene family, counteract the functions of the SWI/SNF multisubunit (20) and repress gene transcription. Recently, evidence has emerged showing that perturbation of complexes that remodel the structure of chromatin by mobilizing nucleosomes may have a key role in tumor suppression and transformation (21).

**Noncoding RNA**

RNA interference (RNAi) is the process by which small, double-stranded RNA molecules (small interfering RNA, or siRNA) can initiate sequence-specific, posttranscriptional gene silencing (22). In addition to posttranscriptional gene silencing, siRNA have recently been shown to mediate transcriptional gene silencing in human cells through an RNA interference-based mechanism (22). The mechanisms of RNAi-mediated epigenetic or transcriptional gene silencing have only recently been suggested and are not fully understood. However, RNAi-mediated
transcriptional gene silencing has been found to result in widespread gene silencing by the recruitment of enzymes that methylate the core histone H3, which in turn leads to recruitment of other proteins that are required to spread the silenced form of chromatin (23). Clearly, such mechanisms are likely to be involved in the development of cancer by silencing certain genes, including tumor suppressor genes.

**EFFECT OF DIET ON EPIGENETIC PROCESSES**

Several observations point to the ability of several environmental factors, including diet, to be key regulators of epigenetic processes, and evidence exists for diet effects on both DNA methylation and histone posttranslational modulation. Diet can influence the degree of methylation by influencing the availability of methyl donors, including folate, choline, and methionine, as well as methyltransferase activity (24–26). Several dietary factors ranging from alcohol to zinc have been reported to influence the supply of methyl groups for the formation of SAM and thereby affect the methylation of CpGs (24). Furthermore, it has been hypothesized that diet might affect DNA demethylation activity, but little is known about such in vivo activity (24). Observations also suggest that DNA methylation patterns may influence the response to a bioactive food component (27). A classic example of the effect of diet in DNA methylation and cancer is the finding that dietary methyl deficiency (of folate, choline, and methionine) in a rat model was shown to alter hepatic DNA methylation patterns and induce hepatocarcinogenesis in the absence of a carcinogen (28). Thus, epigenetic regulation is fundamental to the regulation of cellular proliferation and apoptosis. A more recent report suggests that only with early (9 wk versus 18, 24, or 36 wk of methyl deficiency) refeeding of a methyl-sufficient diet during methyl-deficiency-induced hepatocarcinogenesis are the altered DNA methylation defects reversible (29); which suggests that timing needs to be considered in any intervention.

Recent investigations suggest that butyrate, diallyl disulfide, and sulforaphane can inhibit histone deacetylase enzymes (30) and alter the expression of specific genes. More specifically, Dashwood et al (30) suggested that these dietary constituents may lead to conformational changes in the active site of HDAC, thus leading to its inactivation. Such changes suggest that continual exposure to these bioactive food components is needed to maintain a change in this controlling step in epigenetics. Although acetylation has been shown to be modified by a few food components, and recent studies have found diet to affect modification of histone methylation (26) and histone biotinylation (31), additional areas to examine include the role of bioactive food components in histone phosphorylation, sumoylation, or ubiquitination.

Dietary modulation was recently shown to influence microRNA expression, which suggests subsequent functional changes, including modification of noncoding RNA transcriptional and posttranscriptional gene silencing. In one example, investigators compared the microRNA expression profile during methyl-deficiency-induced hepatocarcinogenesis with the profile of livers from age-matched rats consuming a normal diet (32). The findings indicated that whereas certain microRNA genes were up-regulated, others were down-regulated in tumor tissue compared with normal tissue. An abundant liver-specific microRNA, miR-122, in normal liver tissue was down-regulated in hepatocellular carcinoma of both rodent tissue and human tumor samples. These findings suggest that the down-regulation of miR-122 is associated with hepatocarcinogenesis and could be a potential biomarker for liver cancers. The subsequent effects that follow from alteration of the microRNA profile by diet or bioactive food components on function, such as RNAi-mediated posttranscriptional or transcriptional gene silencing, the process implicated as an epigenetic mechanism, have yet to be reported.

The following discussion provides additional examples concerning the influence of diet on epigenetic processes. More specifically, these examples highlight how an epigenetic-diet interaction early in life may affect metabolic syndrome or its symptoms later in life.

**In utero low-protein diet affects DNA methylation and later disease**

Animal models have been used in an attempt to understand the molecular basis of the relation between early growth restriction and the development of adult disease. For example, the maternal reduced-protein rat model has been used to examine the importance of the maternal environment in determining susceptibility to adult disease (33). In this model, pregnant and lactating rat dams are fed a diet containing 80 g protein/kg, compared with a basal level of 180 g protein/kg, which leads to growth restriction in utero. Offspring of low-protein dams have increased susceptibility to diabetes, insulin resistance, and hypertension when fed a high-fat diet that promotes obesity (34). Several potential mechanisms have been highlighted to describe such resulting metabolic disturbances, such as altered glucocorticoid programming and leptin signaling abnormalities (34). Another study implicated epigenetic alterations (35) through modifications in one-carbon metabolism and methyl supply. In that study, investigators found that changes in methionine metabolism increased homocysteine production, which led to changes in DNA methylation in the fetus (35). Specifically, these investigators found that endogenous methylation of DNA was greater in the livers, but not in kidney or heart tissue, of fetuses from dams fed the 9%-protein diets than in livers from controls (fetuses from dams fed 18%-protein diets). It can be hypothesized that the increased DNA methylation in the liver is the result of some methylation or one-carbon metabolism disturbance, which could influence the silencing of growth-promoting genes during an influential developmental period. Thus, an increase in maternal homocysteine may compromise fetal development, leading to the onset of glucose intolerance and hypertension in adult life through an epigenetic alteration.

A low-protein maternal diet has also been reported to influence the promoter methylation status and expression of the glucocorticoid receptor (GR) and peroxisomal proliferator-activated receptor (PPAR) genes in liver of the offspring (36). Both GR and PPARs were investigated because of their importance for normal embryogenesis and because of their involvement in either the regulation of blood pressure (GR) or lipid and carbohydrate homeostasis (PPARs) in adults (36). In this investigation, rat dams were fed a control protein (18% protein plus 1 mg/kg folic acid), restricted protein (9% protein plus 1 mg/kg folic acid), or restricted protein plus 5 mg/kg folic acid diet throughout pregnancy. During lactation, dams were fed an AIN-76 diet and pups were weaned onto this diet 28 d after birth and killed at 34 d. PPARα but not PPARγ gene methylation was found to be significantly lower and mRNA expression was 10.5-fold higher in
restricted compared with control pups. Additionally, GR gene methylation was also significantly lower and mRNA expression 3-fold higher in restricted compared with controls pups. Interestingly, the restricted plus folic acid diet prevented these changes, which suggests that a one-carbon metabolism defect was involved in the epigenetic alterations in protein-deficient animals. That the hypomethylation of PPARα and GR persisted after weaning when the influence of maternal diet had ceased suggests a mechanism by which insults in early life may lead to persistent changes to the phenotype of the offspring. Increased expression of PPARα and GR could alter factors and other genes to influence the onset of metabolic syndrome or its symptoms.

Increased glucocorticoid exposure during early life has been implicated in the induction of hypertension (37), and altered PPAR activity is associated with the induction of dyslipidemia (36). Furthermore, activation of PPARα in liver may promote a program of lipid-induced activation of genes encoding proteins involved in fatty acid uptake, β-oxidation, fatty acid transport into peroxisomes, and β-oxidation of unsaturated fatty acids (38). Thus, studies of dietary limitation or deficiency have provided some evidence to support an epigenetic-diet interaction affecting alterations early in life, but more study is needed as to how such modulation affects disease in adult animals.

Studies in the agouti model: methyl donor supplementation and genistein

Evidence using the viable yellow agouti (A<sup>V</sup>) model suggests that dietary supplementation in utero can also lead to changes in DNA methylation and to profound effects on the phenotype of the offspring (39–42). In A<sup>V</sup> mice, an endogenous retrovirus-like transposon sequence is inserted close to the gene coding for the agouti protein (43). Normally, a cryptic promoter within the retrotransposon is silenced by methylation, which allows normal tissue-specific and regulated agouti expression. However, if this site is undermethylated, the promoter is active and drives constitutive ectopic expression of the agouti gene, leading to yellow coat color and obesity (and other symptoms of the metabolic syndrome). Dietary supplementation with folic acid, vitamin B-12, choline, betaine, and zinc to maternal diets was correlated with a change in coat color from a yellow to an agouti coat, which is usually associated with lower risks of cancer, diabetes, and obesity and a prolonged life compared with yellow mice (39, 42). Furthermore, these investigators examined DNA methylation in representative phenotypes and found that the representative yellow mice displayed more hypomethylated long terminal repeats 5′ of the agouti gene; in representative agouti or brown coat mice, there was a greater degree of hypermethylation in the long terminal repeat 5′ of the agouti gene. It is important to note that these nutrients were added to a diet considered to be nutritionally adequate. It remains to be determined which component or combination is required to bring about this effect.

Another research team has also examined the effect of methyl donor supplementation (folic acid, vitamin B-12, choline, and betaine) to the maternal diet without zinc in the agouti model and found similar effects on the phenotypic expression of the offspring (40). This team characterized the distribution shift in coat color and CpG methylation of the agouti locus with methyl supplementation compared with that in unsupplemented animals. They found that changes in pigmentation of mouse pup hair coat, ranging from yellow to brown, were significantly associated with supplementation of the pregnant mother’s diet. Furthermore, these coat color changes were directly linked to alterations in DNA methylation with a distribution shift toward increased CpG methylation at the A<sup>V</sup> locus with methyl supplementation. Moreover, by a comparison of tail DNA at 21 d and liver DNA at 100 d, these investigators found that the coat color phenotype and A<sup>V</sup> methylation relation persisted into adulthood.

In a more recent study by Dolinoy et al (41), similar alterations in coat color were induced through maternal ingestion of genistein, the major phytoestrogen in soy, at doses comparable with those a human might receive through a high-soy diet. Most interestingly, the methylation changes at the A<sup>V</sup> locus with genistein supplementation appeared to protect the mouse offspring against obesity in adulthood, which suggests that maternal dietary supplementation is associated with not only altered fetal methylation patterns but also methylation-dependent susceptibility to disease. The mechanism of how genistein affects methylation and epigenetic pathways has yet to be determined, but it is thought to be independent of the one-carbon pathway because no association between genistein supplementation and SAM or S-adenosylhomocysteine concentrations were found. Changes in specific nuclear transcription factors are a logical site of action of genistein, but again, more research is desperately needed in this area to confirm and identify the specific site of action of genistein.

It is important to clarify how observations in the agouti model may relate to human disease. Although the A<sup>V</sup> locus, a retrovirus-like transposon sequence, is not found in the human genome, it is possible that metastable epialleles associated with other transposable elements could similarly be influenced by methylation via in utero exposure to dietary factors. Whitelaw and Martin have proposed that transposable elements in the mammalian genome cause considerable phenotypic variability, making each individual mammal a “compound epigenetic mosaic” (44). Whether such an epigenetic mosaic can be modulated by early diet and how such phenotypes alter susceptibility to chronic disease in adulthood require further study.

Imprinted gene influenced by postweaning diet

Genomic imprinting is an epigenetic modification that inactivates one allele of a gene in a parent-of-origin-dependent manner. It occurs primarily by allelic-specific methylation of cytosines in CpG dinucleotides during gametogenesis. Although relatively few in number, imprinted genes are thought to be vital to normal human development, and their dysregulation appears to affect the risk of developing some diseases (45). One example by which early dietary exposures may affect genomic imprinting involves the imprinted gene insulin-like growth factor 2 (IGF2) (14). The maternally inherited allele of the imprinted gene encoding IGF-II is normally epigenetically silenced, resulting in expression almost exclusively from the paternal allele. Interestingly, loss of imprinting (LOI) at the IGF2 locus has been shown to induce biallelic expression of this mitogenic growth factor in 10% of normal human adults (46) and is implicated in several types of cancer and in Beckwith–Wiedemann syndrome (45), a disease with an array of defects, including several phenotypic and metabolic abnormalities. For the most part, the cause of the IGF2 LOI is unknown. Regardless, mice weaned on either a synthetic control or a synthetic methyl donor-cofactor deficient diet compared with a standard natural ingredient control diet displayed hypomethylation of IGF2 and consequent dysregulation of IGF2 allelic expression in kidney tissue (14). Furthermore, the LOI
caused by the deficient diet persisted during a subsequent 100-d recuperation period after the mice were switched to the control diet. Thus, early nutritional influences may stimulate changes in cytosine methylation to which imprinted genes, such as IGF2, may be susceptible. These are tantalizing findings in view of the relation between IGF-II, insulin, and metabolic syndrome, and they suggest that early nutrition may influence susceptibility to adult obesity, diabetes, cardiovascular disease, and cancer.

METABOLIC CLUES FROM CALORIE-RESTRICTION STUDIES

Calorie restriction (CR) is a dietary regimen that has been shown to extend life span in many organisms, including mammals (47). CR has been shown to induce metabolic changes in rodents, such as decreasing blood glucose, insulin, glycogen, fat, and body weight and improving insulin sensitivity. Mechanistic clues from CR studies may provide insight on how metabolic syndrome might influence the modulation of epigenetic processes and thus affect gene stability and expression and disease. In this regard, a gene that has been found to be overexpressed during CR in yeast is the silent information regulator 2 (Sir2), which is an NAD$^+$-dependent histone deacetylase (or class III HDAC) (47). During CR in yeast, the SIR2 protein is thought to promote transcriptional silencing and genomic stability by deacetylating histones and stabilizing repetitive DNA (48). Mammalian homologues of Sir2 have been identified; mammalian SIRT1 likely modulates key transcription factors in stress response, senescence, and metabolism but appears to have no role in genomic stability (48). However, the mammalian gene SIRT6 appears to be involved in DNA repair pathways and promotes genomic stability and longevity. Investigators have recently shown that inactivation of SIRT6 causes genome instability and premature aging in a mouse model (49). An interesting question to examine is whether diminished SIRT6 expression or regulation results in metabolic syndrome and how this affects transcriptional activity, genomic instability, and cancer risk. Research on the class III HDACs may provide information about their involvement in the various disturbances of the metabolic syndrome and later health outcomes. Interestingly, the red wine polyphenol resveratrol has been found to induce longevity in C. elegans through the action of Sir2 (50). How bioactive food components such as resveratrol influence or mimic the mechanistic and metabolic response of CR in mammalian tissue is another potential area of investigation.

SUMMARY

The probability of epigenetic modulation as a factor in precipitating the metabolic syndrome (or its symptoms) and the inverse, ie, how metabolic syndrome affects epigenetic modulation, are emerging areas of interest in cancer research. A host of studies have shown that genomics and early diet can both influence health outcomes later in life; more recently, studies examining diet-gene and diet-epigenetic interactions have provided additional clues about the early influences of epigenetics. In this regard, both dietary limitations (low protein) and dietary supplementation (methyl donors, genistein) have been found to modify methylation processes that may affect obesity and disease susceptibility later in life. Interestingly, genomic imprinting may be modifiable not only in utero but also after birth, as was observed in a study that found that altering the postweaning diet affected the expression of IGF2, an imprinted gene. Such findings may also help to explain the effect of early-life cellular events on later expression of disease. A greater understanding of the genesis of the metabolic syndrome and its connections with cancer may arise from examining signaling pathways in models of caloric restriction. In fact, examination of common signaling pathways that influence both metabolic syndrome and cancer may provide new avenues to explore. As an example, additional understanding about the link between metabolic syndrome and cancer may be obtained through comparison of hypermethylated and silenced genes in tumors and underexpressed, deregulated, and repressed genes that have also been shown to be involved in the metabolic syndrome. Overall, more probing mechanistic studies aimed at examining the impact of early diet on metabolic phenotype in adult life are needed to unravel the interrelations among diet, epigenetics, metabolic syndrome, and cancer.

The contributions of the authors were as follows: SAR wrote the manuscript, and JAM conceptualized the original idea for the study and contributed by editing the manuscript. The authors did not have any conflicts of interest.

REFERENCES


