COMMENTARY

N-Nitrosodimethylamine in the Kashmiri Diet and Possible Roles in the High Incidence of Gastrointestinal Cancers

Naveed A Chikan[1], Nadeem Shabir[2], Sheikh Shaffi[2], Manzoor R Mir[2], Trupti N Patel[1]*

Abstract

The Kashmiri population is culturally distinct with special dietary features owing to the temperate climatic conditions of Kashmir valley. This has habituated the population to preserve food in smoked, pickled and sundried forms which include considerable amounts of *N*-nitroso compounds (NOCs). These are known to cause cytotoxicity, DNA damage, mutation, unscheduled DNA synthesis and DNA methylation. All of these changes at molecular level are known to contribute to the pathogenesis of cancer. One of the prominent NOCs found in Kashmiri food is *N*-Nitrosodimethylamine (NDMA). Here we review the occurrence of NDMA in sundried foods, dried fish, kehwa, traditional pickle, *Brassica oleracia* and *tobbaco*. We also discuss its possible role in the high prevalence of gastrointestinal cancers in Kashmir.

Keywords: Kashmiri dietary habits - nitroso compounds -N-Nitrosodimethylamine - gastrointestinal cancers

Asian Pacific J Cancer Prev, **13**, 1077-1079

Introduction

Nitroso compounds (NOCs) have been classified as potent human carcinogens by the International Agency for Research on Cancer (IARC 2010). Human exposure to NOCs occurs almost entirely through food and endogenous nitrosation (A process of converting organic compounds into nitroso derivatives) of its precursors in the gastrointestinal tract (Tricker 1997) following absorption into the bloodstream. NOCs may also have deleterious effects in tissues of direct and indirect contact. Epidemiological studies have indeed associated human (endogenous) NOC exposure to several types of cancers including esophagus, stomach, colorectal and bladder cancer (Mirvish et al., 1995; Bingham et al., 1996; Magee et al., 1989). The Kashmiri food contains significant amount of NOCs and NDMA is one of the most prominent Nitroso compounds in this food (Siddiqi et al., 1988).

NDMA the simplest and most widely occurring nitrosamine, is reported to be acute hepatotoxic and potentially carcinogenic in various animal species including humans (Lai et al., 1980; Robbiano et al., 1996; IARC 1998). Studies have described the ability of NDMA to induce DNA damage in the primary culture of human hepatocytes (Martelli et al., 1985). It is commonly inferred that nitrosamines are related to cancer induction through the formation of mutagenic DNA methyl adducts in important genes involved in carcinogenesis (Goto et al., 1999). There is mounting evidence that DNA alkylation, especially the formation of O^6-alkylguanine (O^6-alkG) is involved in mutagenesis and cancer (Doniger et al., 1985; Singer et al., 1991). O^6-Methylguanine (O^6-meG), for instance, has been detected in the DNA of esophageal tissues collected from people living in an area of China where the risk of esophageal cancer is considerably high (Huh et al., 1985).

The aim of this review was to look in to the possible etiology of high incidences of gastrointestinal cancer in Kashmir considering their dietary habits.

Gastrointestinal Cancers in Kashmir

Various studies have been done on incidence rate and distribution of gastrointestinal cancers in Kashmir (Azra & Jan, 1990; Khuroo et al., 1992; Dhar et al., 1993; Makhdoomi et al., 2005; Qurieshi et al., 2011). These studies have shown that cancers of oesophagus and stomach are common in Kashmir and together account for more than 60% of all the cancers prevalent in the valley. In a study conducted by Dhar and his team (1993), it was observed that oesophago-gastric malignancies constitute around 57.5% of total malignancies. In other study (Makhdoomi et al., 2005) based on the biopsy samples of patients collected at Department of Pathology, Govt. Medical College, Srinagar - Kashmir, over a period of 15 years (1984-1998), showed that out of a total of 5892 patients with various malignancies 43.43% were afflicted by gastrointestinal malignancies and of these 76% had

*[1]Division of Medical Biotechnology, School of Bioscience and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India, [2]Division of Veterinary Biochemistry, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar-190006, Jammu and Kashmir, India *For correspondence: dr.tnpatel@gmail.com; tnpatel@vit.ac.in*

oesophago-gastric malignancies. This report shows an alarmingly high percentage of gastric and esophageal cancers in the population under study.

Kashmiri Diet and Nitroso Compounds

Kashmiri population is ethnically very distinct due to their special dietary intakes. Owing to the temperate climatic conditions of Kashmir valley, the population is habituated to preserve foods in smoked, pickled and sundried form. In a study (Siddiqi et al., 1988) it was found that these food products contain considerable amounts of NOCs. Some of the prominent NOCs found in the local food are *N*-Nitrosodimethylamine (NDMA), Methyl Nitrosourea (NMU), *N*-Nitrosopyrrolidine (NPYR), *N*-Nitrosopiperidine (NPip) and *N*-Nitrosoproline (NPro). Further the authors showed that under stimulated gastric conditions there is formation of high concentration of NOCs in the gastric fluids and NDMA being the major one of all, is formed from intake of dried fish (20 μg/kg), dried and pickled vegetables (35.6 μg/kg and 7.3 μg/kg), locally grown *Brassica oleracea* ('Hak') leaves (69.9 μg/kg), and the traditional tea 'Kehwa' (9.2 μg/kg). *Brassica*, a green vegetable from Kashmir, which is a major constituent of the local diet, when cooked contains around 11 μg/kg of NDMA (Kumar et al., 1990). According to the ATSDR (1989) report, LD_{50} of NDMA in rats is 37mg/kg body weight. Repeated intake of food containing high amounts of NDMA could lead to adverse effects of this toxic compound and its metabolites in various organs leading to favorable milieu for cancer especially malignancies of alimentary canal. Table 1. shows the common Kashmiri food products and different types of NOCs present in these diets.

Several researchers (Khuroo et al., 1992; Dhar et al., 1993; Murtaza et al., 2006; Qurieshi et al., 2011) have concluded a probable role of dietary factors in high incidence of gastrointestinal cancers in Kashmir. Khuroo with his colleagues (1992) were the first to link special dietary habits of Kashmiris with high incidence of esophageal cancer. Dhar et al. (1993) concluded that preponderance of esophageal cancer was attributable to the local practice of drinking boiling hot salted tea. Murtaza et al. (2006) reviewed the dietary habits of three groups consisting of 51 individuals each, in Kashmiri population. In this study Group I represented patients of esophageal cancer; Group II represented control with familial history of gastric cancer and Group III were control with no history of gastric cancer. A significant agreement of dietary habit and varying degree of incidence of esophageal cancer post consumption of locally grown Brassica vegetable (Hakh), red chillies, hot salty soda tea, and local baked bread was observed. A very recent survey by Qurieshi and co-workers (2011) on 81 patients of gastric carcinoma in Government Medical College, Srinagar – Kashmir, reported that 87.35% of these patients consumed sun dried vegetables; 48% had sun dried fish; 66.7% consumed pickles and 35.8% of them had smoked fish in their regular diets. All of these consumables are known to contain variable amounts of NDMA (Siddiqi et al., 1988).

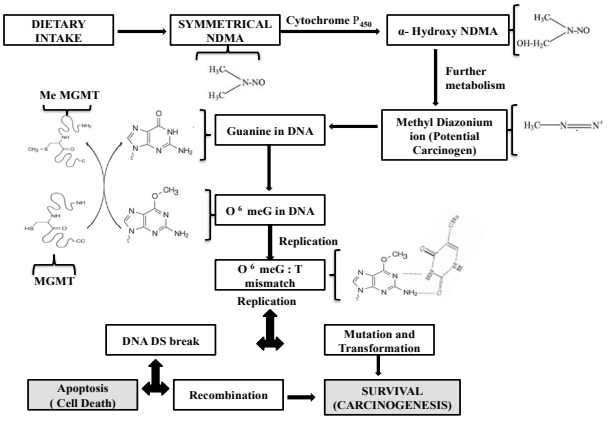

Figure 1. Molecular Mechanism of NDMA Induced Carcinogenesis.

Possible Role of NDMA in Etiology of Cancers

N-Nitrosodimethylamine is a symmetric *N*-nitrosamine and hydroxylation of either of the two carbons will yield methylating agent - Alpha hydroxyl NDMA. For this it undergoes enzymatic oxidation through Cytochrome -P_{450} to convert into a precursor (α- hydroxyl NDMA) which finally metabolizes into an ultimate carcinogen - methyl diazonium ion ($CH_3N_2^+$) (Magee et al., 1962; Wang et al., 2002; Agnew et al., 2004). *In vivo* studies on rat liver showed alterations of DNA methylation patterns by NDMA (Singer & Grunberger, 1983) and it was found that 6.6% of O^6 position in guanine is methylated. Methylation at the guanine O^6 (O^6-methyl-guanine - O^6 meG) position confers a high mutagenic and carcinogenic susceptibility. DNA polymerases insert thymine opposite O^6-methyl-guanine with a similar frequency of normal cytosine opposite guanine (Warren et al., 2006). Flow chart (Figure

Table 1. Showing Typical Food Products Consumed by Kashmiri Population and Type of NOCs Contained in These Diet

Type of Food Products	Description	*N*-Nitroso compound
Sundried foods	Different vegetables sundried in summers and consumed over a period of time during different seasons	NDMA
Dried fish	Fresh water fish dried in sun and available in the markets; they are consumed over a period of time	NDMA
Kehwa	Traditional sweet drink , with no tea	NDMA
Traditional pickle	Mixed vegetable fermented with specific spices and consumed all round the year	NDMA
Brassica oleracia	Commonly used green leafy vegetable.	NDMA & NMU
Tobacco	In the form of cigarette/Hubble bubble - Hukka	NDMA, NPYR & NPIP
Salt Tea	Tea treated with bicarbonate, brewed at high temperature and diluted for drinking	NPRO & NPIC

1) shows a diagrammatic representation of molecular events taking place during NDMA induced mutagenesis and thus leading to onset or progression of cancer.

Conclusion

To validate the role NDMA in the etiology of gastrointestinal cancers in Kashmir valley a high throughput Genome wide association studies (GWAS) would give a better understanding to the molecular mechanisms contributing to gastric cancers. Looking at GC - AT transitions per se in promoter regions and exon of important genes contributing to gastrointestinal cancers would help us explore novel target genes for early diagnosis and therapeutics. Families with history of gastrointestinal cancers should totally evade the use of food rich in NOCs. The most important factor working towards the control of gastrointestinal cancer in Kashmiri population is to bring awareness of dietary intakes and reduce the intake of NOC containing food products.

Acknowledgements

This review was supported by VIT University Vellore-632014, Tamilnadu and the literature available at the VIT Library .The authors declare that they have no competing interests.

References

Agnew TE, Kim HJ, Fishbein JC (2004) . Diazonium ion chemistry: replacement of H by alkyl at the central carbon accelerates an *S*N2 substitution reaction. *J Phys Org Chem*, **17**, 483-8.

ATSDR (1989). Toxicological profile for *N*-Nitrosodimethylamine. U.S. Public Health Service.

Bingham SA, Pignatelli B, Pollock JR, et al (1996). Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis*, **17**, 515-23.

Dhar GM, Shah GN, Naheed B, et al (1993). Epidemiological trend in the distribution of cancer in Kashmir Valley. *J Epideniol Community Health*, **47**, 290-2.

Doniger J, Day RS, Dipaolo JA (1985). Quantitative assessment of the role of O6-methylguanine in the initiation of carcinogenesis by methylating agents. *Proc Natl Acad Sci USA*, **82**, 421-5.

Fleisher AS, Esteller M, ONE AUTHOR, et al (1999). Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. *Cancer Res*, **59**, 1090-5.

Goto Y, MatsudaT, Ito K, et al (1999). Mutagenicities of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in Drosophila and their relationship to the levels of O-alkyl adducts in DNA. *Mut Res*, **425**, 125-34.

Huh N, Satoh MS, Shiga J, et al (1989). Immunoanalytical detection of O4-ethylthymine in liver DNA of individuals with or without malignant tumours. *Cancer Res*, **49**, 93-7.

IARC (2010). Agents Classified by the IARC Monographs, vol. 1–100.

IARC (1998)Monographs on the Evaluation of Carcinogenic Risks to Humans. Some N- Nitroso Compounds. Volume 17

Khuroo MS, Zargar SA, Mahajan R, et al (1992). High incidence of esophageal and gastric cancer in Kashmir in a population with special personal and dietary habits. *Gut*, **33**, 11-5.

Kumar R, Mende P, Tricker AR, et al (1990). N-Nitroso compounds and their precursors in *Brassica oleracea*. *Cancer Lett*, **54**, 61-5.

Lai DY, Arcos JC (1980) . Dialkylnitrosamine bioactivation and carcinogenesis. *Life Sci*, **27**, 2149-265

Makhdoomi R, Khan AR, Khurshid N, et al (2005). The Changing Pattern Of Oesophago-Gastric Cancer. *JK Pract*, **12**, 189-92.

Tricker AR (1997). *N*-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. *Eur J Cancer Prev*, **6**, 226-68.

Magee PN (1989). The experimental basis for the role of nitroso compounds in human cancer. *Cancer Surv*, **8**, 207-39.

Magee PN, Farber E (1962). Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine *in vivo*. *Biochem J*, **83**, 114-24.

Martelli A, Robbiano L, Giuliano L, et al (1985). DNA fragmentation by *N*-nitrosodimethylamine and methyl methanesulfonate in human hepatocyte primary cultures. *Mutat Res*, **144**, 209-11.

Mirvish SS (1995). Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett*, **93**, 17-48.

Murtaza I, Mushtaq D, Margoob MA (2006). A study on *p53* gene alterations in esophageal squamous cell carcinoma and their correlation to common dietary risk factors among population of the Kashmir valley. *World J Gastroenterol*, **12**, 4033-7.

Qurieshi MA, Masoodi MA, Kadla SA, et al (2011). Gastric Cancer in Kashmir. *APJCP*, **12**, 303-7.

Robbiano L, Mereto E, Corbu C, et al (1996). DNA damage induced by seven *N*- nitroso compounds in primary cultures of human and rat kidney cells. *Mutat Res*, **368**, 41-7.

Sawada S, Yamanaka T, Yamatsu K, et al (1991). Chromosome aberrations, micronuclei and sister-chromatid exchanges (SCEs) in rat liver induced *in vivo* by hepatocarcinogens including heterocyclic amines. *Mutat Res*, **251**, 59-69.

Siddiqi M, Tricker AR, Preussmann R (1988). The occurrence of preformed N-nitroso compounds in food samples from a high risk area of esophageal cancer in Kashmir, India. *Cancer Lett*, **39**, 37-43.

Siddiqi M, Tricker AR, Preussmann R (1988). Formation of *N*-nitroso compounds under simulated gastric conditions from Kashmir foodstuffs. *Cancer Lett*, **39**, 259-65

Singer B, Essigmann JM (1991). Site-specific mutagenesis: retro-spective and prospective, *Carcinogenesis*, **12**, 949-55

Umbenhauer D, Wild CP, Montesano R, et al (1985). O^6-Methyldeoxyguanosine in esophageal DNA among individuals at high risk of esophageal cancer. *Int J Cancer*, **36**, 661-5.

Tardiff RG, Lohman PHM, Wogan GN (1994). Methods to Assess DNA Damage and Repair. lnterspecies Comparisons, SCOPE Published by John Wiley & Sons Ltd

Verbeek B, Southgate TD, Gilham DE, et al (2008). O^6-Methylguanine-DNA methyltransferase inactivation and chemotherapy. *Br Med Bull*, **85**, 17-33

Wang PG, Xian M, Tang X, et al (2002) . Nitric oxide donors: chemical activities and biological applications. *Chem Rev*, **102**, 1091.

Warren JJ, Forsberg LJ, and Beese LS (2006).The structural basis for the mutagenicity of O6-methyl-guanine lesions . *PNAS*, **103**, 19701-6