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RESEARCH ARTICLE

MEAT PRODUCTS AS AN ENGENDER OF PROSTATE CANCER

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ABSTRACT

High-temperature cooked meat contains NOCs, heterocyclic amines and polycyclic aromatic hydrocarbons. In rodents, a high intake of such compounds induces prostate tumours. In this article the association between heterocyclic amines, a common meat mutagen and prostate cancer is studied. The normal cells from the prostate gland are extracted and are treated with the above mentioned mutagens to study the cell viability, proliferation and the effects of the mutagens on the chromosomes. The cells showing increased proliferation rate and showing some chromosomal aberration in the metaphase plate indicates its conversion from normal to cancerous cells. The rate of conversion of normal cells to cancerous cells by a specific mutagen determines its mutagen city.

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INTRODUCTION

Prostate cancer has become a one of the major cause of mortality globally during the last few decades in men. It is the second most frequently diagnosed cancer in men worldwide and the fifth most common cancer overall with more than three quarter of cases occurring in men above 65 years of age. Approximately 4.04 million lives are lost globally due to prostate cancer. Prostate cancer is expected to be a leading cause of death in men by 2020. The incidence of prostate cancer has shown significant variation across the globe. The highest rates of prostate cancer are reported in Australia/New Zealand (104.2/100,000), Western and Northern Europe, and North America. In the Asian countries, prostate cancer incidence has been reported to vary from 3.0/100,000 in Iran to 20.3/100,000 in the Philippines; the highest reported cases in the year 2000 (Garcia *et al.,* 2007). Data from National Cancer Registries show that incidence of certain types of cancer are on rise in India, prostate cancer being one of them. Prostate is the second leading site of cancer attack in males in large Indian cities like Delhi, Kolkata, Pune and Trivandrum, third leading site of cancer in cities like Bengaluru and Mumbai. It is also ranked among the top ten leading sites of cancers in the rest of the population based cancer registries (PBCRs) of India. The PBCRs at the states like Bangalore (Annual Percentage Change: 3.4%), Chennai (4.2%), Delhi (3.3%), Mumbai (0.9%) and Kamrup Urban District (11.6%) portrayed a statistically significant increasing trend of incidence rates of prostate cancer over time. Of all prostate cancers, 85% were detected late (stages III and IV) in India in contrast to the United States where only 15% were diagnosed in the late stages. This reasons the increasing number of deaths due to prostate cancer in India as this cancer is fatal once it reaches the malignant stages (Stage III and Stage IV) and attacks the bones and other organs (Lalitha *et al.,* 2012). Convincing evidences have establised the relationships between consumption of red meat and an increased risk prostate cancer. According to the WHO, "Red meat refers to all kinds of mammalian muscle meat, including, beef, veal, pork, lamb, mutton, horse, goat." "Processed meat refers to a kind of meat which has been transformed through salting, curing, fermentation, smoking, grilling, charring or other processes to enhance flavour or improve preservation." The WHO classifies these processed red meat into group 1 carcinogen and classifies the red meat as a group 2A carcinogen. A group 1 carcinogen is defined as those which are "carcinogenic to humans" while a group 2A carcinogen are those which "probably" causes or are expected to cause cancer. In a 2017 narrative review, Scientist Wolk concluded that the consumption of approx100 grams of unprocessed red meat per day is associated with an increased risk for advanced prostate cancer (later stages) by 19%. He also reported that consuming 50 grams of processed red meat per day increased the risk for advanced prostate cancer by 4% and cancer mortality by 8% (Perez *et al.,* 2002). Heterocyclic amines (HCAs) are a class of chemicals formed when meat or meat productsare subjected to cooking using high-temperature methods, such as frying or grilling directly over an open flame.

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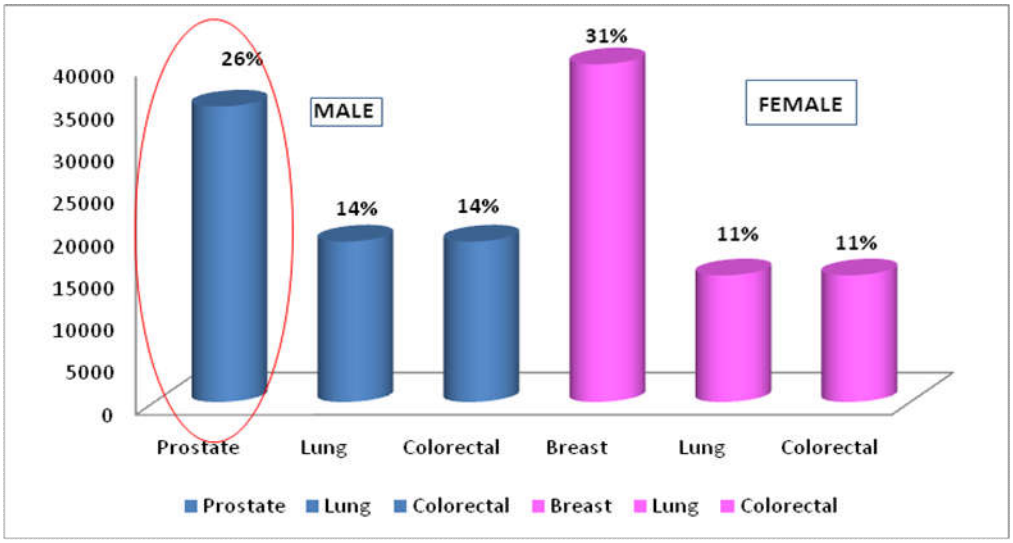

Fig. 1. The above graphs depicts the increasing risks of different cancer in percentage (Blue in male and Pink in females) proving prostate cancer as one of the most growing form of cancer, hence increasing risk for men

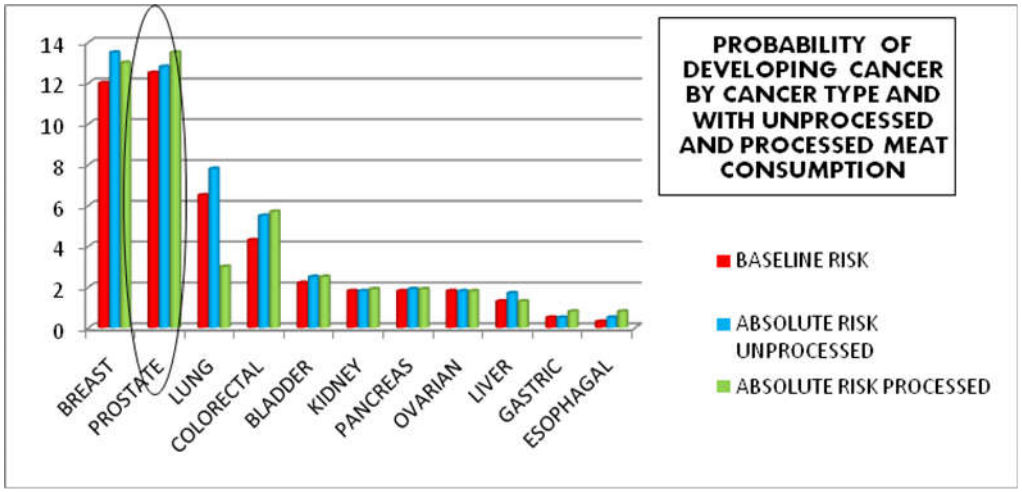

Fig. 2. Probability of developing cancer by cancer type and with unprocessed and processed meat consumption

These heat compounds can hamper the gut and hence are considered possibly carcinogenic by the International Agency for Research on Cancer. HCAs are formed when amino acids, sugars, and creatine or creatinine react at high temperatures. HCAs are capable of damaging the DNA only after some specific enzyme catalysis in the body, a process called“ bio activation.” Studies have found that the activity of these enzymes, which can differ among people, may be relevant to the cancer risks associated with exposure to these compounds. Polycyclic aromatic hydrocarbons (PAHs) are also formed when fat and juices from meat grilled directly over a heated surfaceor open fire drip onto the surface or fire, causing flames and smoke. The smoke contains PAHs that then adhere to the surface of the meat. N-nitroso compounds (NOCs) are also found in processed red meat. The major genes responsible to indicate prostate cancer are tumour suppressor RNASEL gene, DNA mismatch repair MSH2 and MLH1 genes and prostate developing HOXB13 gene. Studying of chromosomal aberration can conclude alterations in these genes as they are directly responsible for prostate cancer. This article mainly deals with the mutagenicity of heterocyclic amines and their role in prostate cancer. The majority of the literature on the topic supports the existence of a link between red meat and cancer, although we cannot conclude causality without intervention studies. Further research is required to establish the mechanisms that could be responsible for an increase in cancer risk, specifically colorectal cancer risk, due to processed and unprocessed red meat consumption.

MATERIALS AND METHODS

Chemicals required for target mutagen preparation (method of Bjeldanes *et al.*)

- 50gm grilled or charred meat sample
- Distilled water
- 0.1M HCl pH 2.0
- 1M NaOH pH 7.0
- Acetone
- Methanol

Chemicals required for in-vitro mammalian cell gene mutation test

- Cell stock culture
- Mammalian cell culture medium (DMEM)
- Target mutagens (heterocyclic amines extracted from meat sample in the previous experiment)

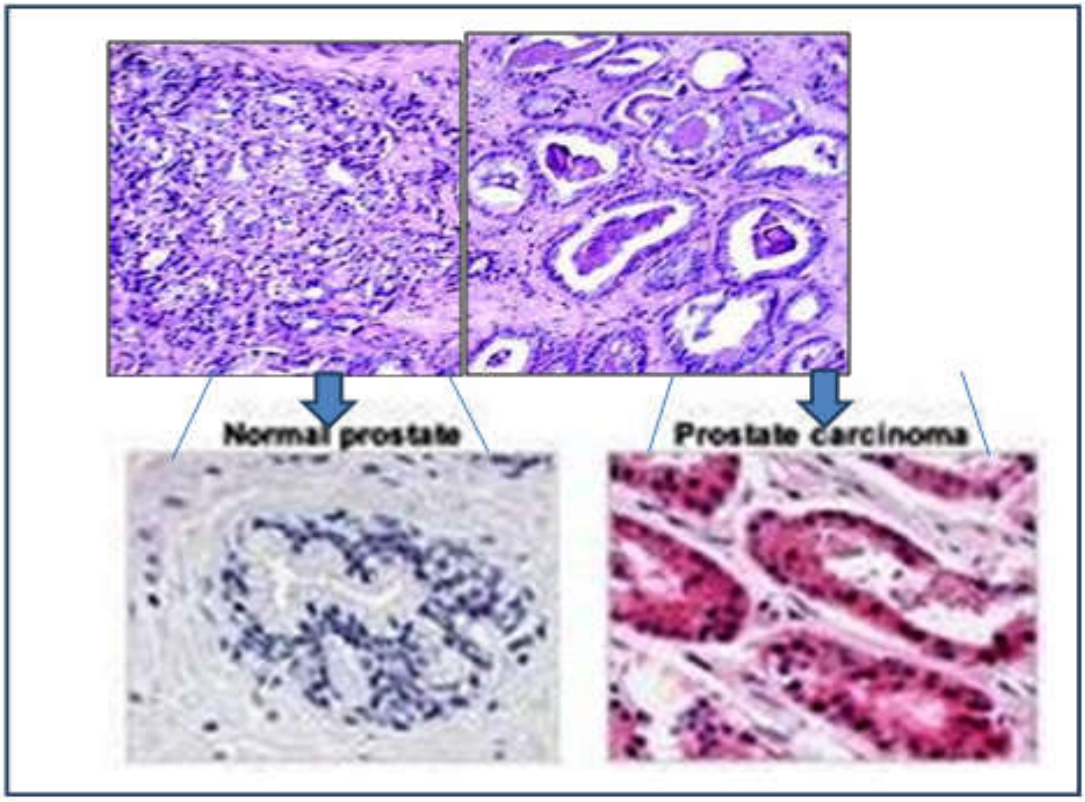

Fig. 3. Morphology of normal and cancerous prostate cells under microscope

Chemicals required for mammalian chromosome aberration test

- Cell culture exposed to test substance
- Colchicine to arrest cells at metaphase stage

Protocol for target mutagen preparation (Method of Bjeldanes *et al.* (1982)

- 50 g of meat sample was taken and homogenised in a double volume of distilled water in a mixer-grinder.
- The homogenate was acidified with 0.1M HCl to pH 2.0 and centrifuged at 6000×g for 15 minutes.
- The supernatant was collected and pellet resuspended in distilled water, acidified and centrifuged again.
- The supernatants were combined and neutralized with 1M NaOH to pH 7.0.
- The cloudy supernatant was filtered through What man filter paper no.1 and clear filtrate applied to a column of Amberlite XAD-2 resin (1.5cm×10cm) at 2 ml/min.
- Then 10 ml of distilled water (pH 7.0) was introduced to the column and the adsorbed compounds finally eluted with 25 ml of acetone followed by 25 ml of methanol.
- Extracts were evaporated to dryness in a vacuum rotary evaporator and resuspended in 1ml methanol and used for further experiments.

Protocol for in-vitro mammalian cell gene mutation test

- Cells are propagated from stock cultures, seeded in culture medium and incubated at 37 degrees.
- Prior to use in this test, cultures may need to be cleansed of pre-existing mutant cells.
- Test substances prepared in the previous experiment are taken to treat the proliferating cells.
- Proliferating cells are exposed to test substance at different concentrations both in presence and absence of an appropriate metabolic activation system.
- Exposure time depends on the mutagen tested. Usually 3-6 hours is effective.
- Cell proliferation (doubling time), viability on selective and non-selective medium are determined.
- Colonies formed are scored based on small and large colonies on at least one concentration of the test mutagen. The colony size and doubling time determines the rate of damage caused to the genes responsible for causing prostate cancer.

Mammalian chromosome aberration test

- Cell culture is exposed to test substance both with and without metabolic activation during about 1.5 normal cell cycle lengths.
- At least 3 analyzable concentrations of the test substance should be used.
- At pre-determined intervals after exposure of cell cultures to the test substance, the cells are treated with colchicine, to restrict the cell division in metaphase stage, followed by harvesting and staining of these cells.
- Metaphase cells in the metaphase plate are analyzed microscopically for the presence of chromosome aberration.

RESULTS AND DISCUSSION

In-Vitro Mammalian cell Culture test: Once the exposure time is completed for the treated cells, their cytology (viability, proliferation and colony size are examined). In case of positive response, treated cells are scored using the criteria of small and

large colonies on at least one concentration of the test mutagen and on the negative or positive controls. The molecular and cytogenetic natures of both large and small colony mutants are explored. Mutant cells which have suffered the most genetic damage have prolonged doubling time and thus form small colonies. This damage typically ranges from the loses of the entire gene(s) to karyotypically visible chromosome aberrations. The induction of small colony mutants has been as associated with chemicals that induce gross chromosomal aberrations. Less seriously affected mutant cells grows at a rate similar to parental cells and form large colonies (Moore *et al.,* 1987).

Mutant frequency= No. of mutant cells/no of surviving cells

Mutant frequency of the test mutagen is determined by seeding known number of cells in medium containing the selective agent to detect the mutant cells and in medium without selective agent to determine cloning efficiency (viability). The mutant frequency is then derived from a number of mutant colonies in selective medium and the number of colonies in non-selective medium

Mammalian Chromosome Aberration Test: The metaphase spread of the treated cells are examined under microscope and compared with the metaphase spread of normal prostate cells. Any chromosomal changes observed might indicate the changes in the tumoursuppressor RNASEL gene, DNA mismatch repair MSH2 and MLH1 genes and prostate developing HOXB13 gene because these genes are directly responsible for prostate cancer. This result is further supported by the small colony size of the treated cells from the previous experiment. This test helps us confirm the effect of the heterocyclic amines on prostate cells in the genetic level[11].

Conclusion and future Prospects: The mechanism of heterocyclic amines inducing prostate cancer is uncertain till date. The strength of this study is its detailed discussion and information about the effects of consumption of charred meat on human health. Heterocyclic amines are a huge class of compounds that accumulate in the charred overcooked meat and over and repeated consumptions of such meat can result in prostate cancer due to accumulation of these compounds in the body followed by their action on the prostate cells. Once the action of this heterocyclic amines are confirmed, PET scan can be performed on the prostate glands of people consuming processed and unprocessed red meat frequently, to ensure the condition of their gland and to confirm the absence of cancerous cells in their gland, because earlier the disease is diagnosed easier it is to cure. Still much research and studies needs to be conducted on prostate cancer as this field still remains untouched.

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REFERENCES

Rastogi T, Devesa S, Mangtani P, Mathew A, Cooper N, Kao R. *et al*. 2008. Cancer incidence rates among South Asians in four geographic regions: India, Singapore, UK and US. *Int J Epidemiol.,* 37:147-60.

Aaron, C.S. and Stankowski, Jr. L.F. 1989. Comparison of the AS52/XPRT and the CHO/HPRT Assays:Evaluation of Six Drug Candidates. Mutation Res., 223, 121-128.

Bjeldanes LF, Grose KR, Davis PH, Stuermer DH, Healy SK, et al. 1982. An XAD-2resin method for efficient extraction of mutagens from fried ground beef. *Mutat Res.,* 105: 43-49.

Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O. *et al*. 2012. International variation in Prostate Cancer incidence and mortality rates. *Eur Uro.,*61:1079-92.

Chu, E.H.Y. and Malling, H.V. 1968. Mammalian Cell Genetics. II. Chemical Induction of Specific LocusMutations in Chinese Hamster Cells *In Vitro*, Proc. Natl. Acad. Sci., USA, 61, 1306-1312.

Garcia M, Jemal A, Ward EM, Center MM, Hao Y, Siegel RL, *et al*. 2007. Global Cancer facts and figures 2007. Atlanta, GA: American Cancer Society.

Lalitha K, Suman G, Pruthvish S, Mathew A, Murthy NS. 2012. Estimation of time trends of incidence of Prostate Cancer-an Indian scenario. *Asian Pac J Cancer Prev.,* 13:6245-50

Liber, H.L. and Thilly, W.G. 1982. Mutation Assay at the Thymidine Kinase Locus in Diploid HumanLymphoblasts. Mutation Res., 94, 467-485.

Moore, M.M., DeMarini, D.M., DeSerres, F.J. and Tindall, K.R. (Eds.) 1987. Banbury Report 28:Mammalian Cell Mutagenesis, Cold Spring Harbor Laboratory, New York.

Moore, M.M., Harington-Brock, K., Doerr, C.L. and Dearfield, K.L. 1989. Differential MutantQuantitation at the Mouse Lymphoma TK and CHO HGPRT Loci. Mutagenesis, 4, 394-403.

Parkin DM, Vatanasapt V. 2001. Cancer registration in Asia in the year 2000 - past, present and future. Asian Pac J Cancer Prev;5:1-89.

Perez C, Lopez De Cerain A., Bello J. 2002. Modulation of mutagenic activity in meat samples after deep-frying in vegetable oils. *Mutagenesis* 17: 63-66.

Scott, D., Galloway, S.M., Marshall, R.R., Ishidate, M., Brusick, D., Ashby, J. and Myhr, B.C. 1991. Genotoxicity Under Extreme Culture Conditions. A report from ICPEMC Task Group 9. Mutation Res.,257, 147-204.

Sim HG, Chang CW. 2005. Changing demography of prostate cancer in Asia. Eur J Cancer;41:834-45.

Tyagi B, Manoharan N, Raina V. 2010. A case control study on prostate cancer in Delhi. *Asian Pac J Cancer Prev.,* 11:397-401.

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