CYP1A1 VARIANT rs2198843 AND CANCER SUSCEPTIBILITY IN HIMACHAL PRADESH POPULATION

Project report submitted in partial fulfilment of the requirement for the Degree of

Bachelor of Technology in Biotechnology

Submitted by

Rakshita (161815) & Veena (161822)

Under the supervision of *Dr. Harish Changotra*

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology,Waknaghat,

Solan-173234, Himachal Pradesh

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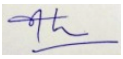
CERTIFICATE

This is to certify that the work titled **"CYP1A1 Variant rs2198843 and Cancer Susceptibility in Himachal Population"**, submitted by Rakshita and Veena in partial fulfilment for the award of degree **of Bachelor of Technology in Biotechnology** of Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.



Signature of Supervisor

Harish Changotra, Ph.D. (Associate Professor)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology Solan,

Himachal Pradesh 173234 INDIA.

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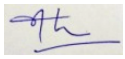
SUMMARY

The term cancer is defined as a disease that is characterized by the uncontrollable and undefined growth of cells. The cancerous cells proliferate in the opposition to normal controls and they have the capability to migrate and occupy the surrounding tissues. Cancer is a disease which is known to be a leading cause of death all around the globe. Many studies are going on and many experiments are being performed in order to find out the main cause of a cell to become cancerous and cause this disease in the body and then leads to death. But still there is no successful treatment of cancer. There are many factors for the development of cancer like lifestyle, environmental, biological, radiations and genetics. The genetic causes involve the defect in genes of an organism which can be due to various reasons. These changes can be transferred (inherited) from parents if these occur in germ line and are in reproductive cells of the human body. The changes that occur are called germ line changes and are present in every cell of the offspring. Some genetic changes causing cancer are also obtained during the lifespan of an individual. The genetic changes that take place after fertilization are called somatic changes.

Here in this study we have been studying single nucleotide polymorphism rs2198843 of CYP1A1 gene to find out its role in the susceptibility of Himachal Pradesh population towards common cancers types. This is a case-control study and the method we are using to find out the polymorphism pattern is Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP).



Signature of Student

(Rakshita; 161815 and Veena; 161822)

Signature of Supervisor

(Dr. Harish Changotra)

Date: _____

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[1] INTRODUCTION

Cancer is said to be a disease in which growth of abnormal cells occurs which tend to multiply in an uncontrollable manner and in some cases spread to other parts of the body. The cells in various parts of body grow normally and have a specified life cycle. However, when these cells in a specific part of the body grow in an unevenly manner, development of cancer takes place [1].There are different varieties of cancer, which all begin due to uncontrolled growth of those cells that are not normal. The cells of cancer are known by some heritable properties: they and including their progeny multiply in defiance of the common restraints on division of cells and occupy the territories that are normally reserved for rest of the cells. The combined action of these cells makes cancer very much dangerous. If the multiplication is not in control, it may form a tumour or we can say it may lead to formation of abnormal cells [2]. The ability of break loose means it causes invasiveness and then through this it enters the vessels of lymphatic system or blood stream and then it forms a tumour known as secondary tumour. This full process is known as metastases, at the rest of the sites in the body. A tumour is a clump of tissue that is made by growth of abnormal cells. There are two specific types of tumour- Malignant & Benign.

Malignant tumours (cancerous) penetrate into the nearby tissues and then enter the blood vessels, and then at last metastasize into different sites. Benign tumours (non-cancerous) are not that much cancerous they grow only locally and cannot spread by invasion or metastasis [3].

There are 2 main classes of genes- Tumour suppressor genes and Proto-oncogenes. These genes play an important role in the beginning and development of cancer.

The function of tumour suppressor gene is to prevent the growth of neoplastic which means the absence of protein (normal) product results in the inactivation and leads to cancer development. When it mutates, the cell grow in an uncontrollable manner and form tumour. For example- BRAC1, BRAC2, p53[4].

Oncogenes is termed as the mutated alleles of wild type genes with the deviant and new ability to develop cancer. They are cellular genes (normal) which helps in controlling differentiation and cell proliferation. This gene take action in dominant way (they are operated by a single allele mutation) while in tumour suppressor genes they need inactivation of both the allele. This disease mainly arises almost in any place in the body [5]. Carcinoma is the most ordinary type of cancer which is approximately 85%. This type begins from the cells that make up internal and external surfaces of the body like epithelial cells. Next is Sarcomas, they are those cancers that comes from those cells which is present in supporting (connective) tissues of the body like muscles, bones etc. Lymphomas are those cancers which arise in several tissues, nodes of lymph in the immune system (body). Last one is Leukaemia's; they are mainly present in the bone marrow.[6]

There are many examples of various types of cancers, Such as Head and Neck Cancer that is mainly found in the mucus (squamous cells) and in some moist regions of throat, mouth, salivary glands, oral cavity, pharynx, nasal cavity. Next is lung cancer means that type of malignant cancer that is found in the lungs and leads to uneven growth of cells and multiplication of cellular masses in the human body. This also spreads to different parts of the body and named as lung carcinoma. The other one more type of cancer, Cervical cancer is found from the cells of cervix. There is an abnormal growth of cells. The cell division also becomes uncontrollable and spreads to rest of the body. e.g. lungs, rectum, liver etc. It means the combined result of precancerous stages for a very long period [7].

Cancer is caused when the loss of normal growth control occurs. In case of normal cells, the proportion of death of old cells and growth of new cells are kept in balance. In case of this disorder, this balance is disarranged. This disarrangement results from the uncontrollable growth of cells or disappearance of cells ability to undergo apoptosis (old cells normally self- destruct) [8].

Like in our body growth maturation occurs naturally and the death of every single cell occurs in our body. The body that is affected by cancer, its detection can be done if there is a tumour in our body but in leukaemia this doesn't happen [9]. The cells that are cancerous losses this mechanism and they continues to grow very fast and rapidly multiples. The uncontrollable growth and the deficiency in the decline phase in the life cycle leading to the division of many cells. This division of cells occurs from very a smaller number of cells within a very less time period. This increase in the growth of cells that occur in our body causes fast drained of the energy in the body that leads to improper functioning of the body and weakness of the immune responses. These irregularities occur because there is a vast fighting for the oxygen and nutrients between the cancerous cells and normal cells. Until very recently, when cloning technologies allowed the isolation of cellular genes from spontaneously arising tumours, the only way to get at cancer-causing genes was to study those present in the genomics of certain DNA and RNA tumour viruses [10].

Most prevalent types of cancers in India

MALE	**FEMALE**
LUNG	LIP, ORAL CAVITY
LIP, ORAL CAVITY	BREAST
COLORECTAL	LUNG
STOMACH	CERVIX
OESOPHAGUS	GASTRIC

SCENARIO OF CANCER WORLDWIDE

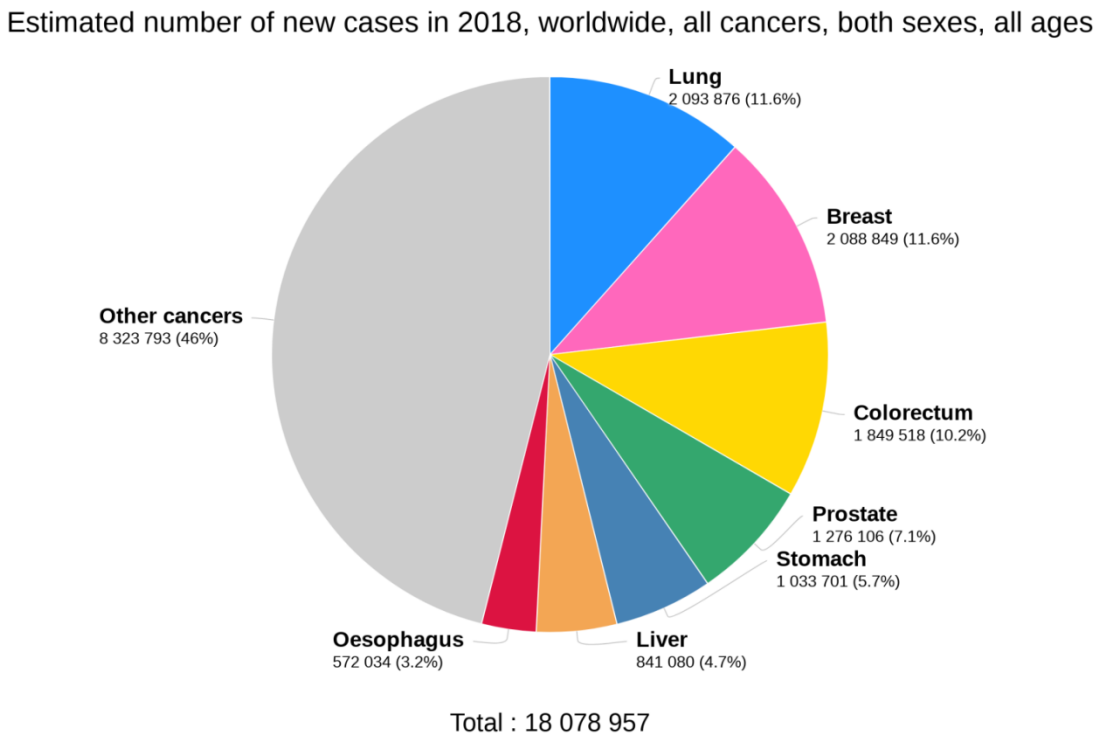


FIGURE 1- Worldwide Cases of Cancer (2018)

Source- cancer.org

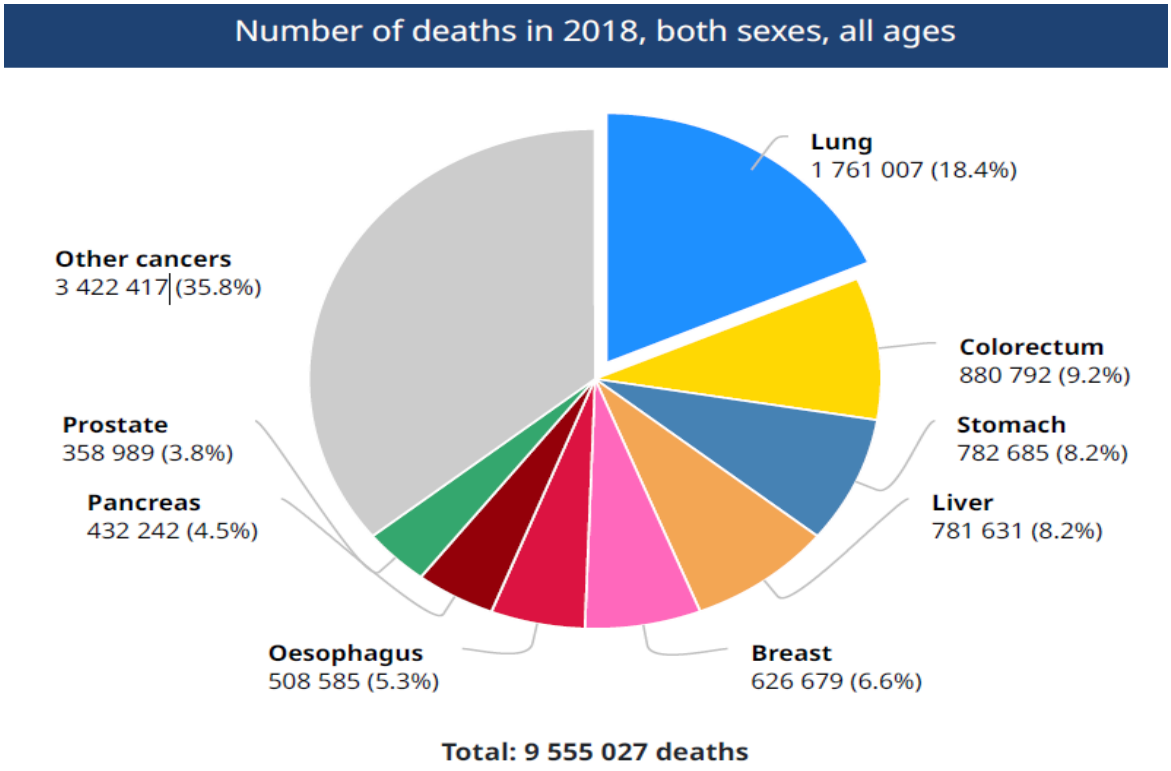


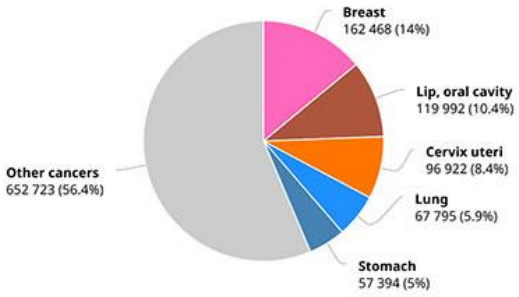
FIGURE 2- Total Number of Deaths due to Cancer (2018)

International Agency for Research on Cancer
World Health Organization

India

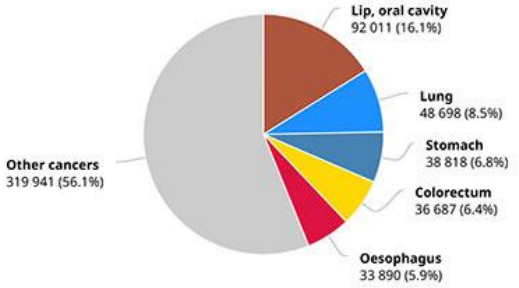
Source: Globocan 2018

Number of new cases in 2018, both sexes, all ages



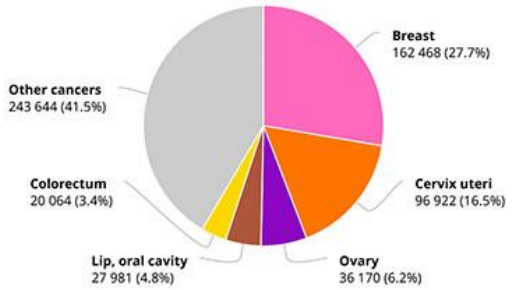
Total: 1 157 294

Number of new cases in 2018, males, all ages



Total: 570 045

Number of new cases in 2018, females, all ages



Total: 587 249

Summary statistic 2018

	Males	Females	Both sexes
Population	701 546 980	652 504 878	1 354 051 855
Number of new cancer cases	570 045	587 249	1 157 294
Age-standardized incidence rate (World)	89.8	90.0	89.4
Risk of developing cancer before the age of 75 years (%)	9.81	9.42	9.58
Number of cancer deaths	413 519	371 302	784 821
Age-standardized mortality rate (World)	65.8	57.5	61.4
Risk of dying from cancer before the age of 75 years (%)	7.34	6.28	6.80
5-year prevalent cases	1 000 485	1 257 723	2 258 208
Top 5 most frequent cancers excluding non-melanoma skin cancer (ranked by cases)	Lip, oral cavity Lung Stomach Colorectum Oesophagus	Breast Cervix uteri Ovary Lip, oral cavity Colorectum	Breast Lip, oral cavity Cervix uteri Lung Stomach

Cancer are caused by some substances called carcinogens. This substance can be a chemical material. Cancer is caused due to those factors which are related to biological, environmental, occupational, viral, some chemicals etc. [11]

LIFESTYLE RELATED FACTORS	Smoking Tobacco, Drinking Alcohol, ultraviolet radiation (sunlight)
BACTERIA AND VIRUSES	HCV, HPV, Epstein-barr virus
SOME DRUGS	Certain hormones, agents of antineoplastic
RADIATIONS	Soil radon, X-ray radiation (Ionising radiation) & UV radiation in sunlight (Non-ionising radiation)
WORK AND LIVING ENVIRONMENT RELATED FACTORS	Pitch and Tar, Some polynuclear hydrocarbons (Benzopyrene), Compounds of metals, Plastic chemicals (Vinyl Chloride)

The main reason for initiation of carcinogenesis and causing cancer is due to mutations and changes in epigenetic in the tumour suppressor genes and oncogenes. Some of other kinds of mutations are mis-sense, frameshift & nonsense mutation. It is not essential that those mutations which are affecting amino acids are the only reason for carcinogenesis but also those mutations that are affecting the sites of splicing and promoters are the cause of cancer [12]. The reasons that leads to mutations are lack of large DNA segments and small DNA segments, translocations, inversions, truncated sequences that is due to looping. The combined result of cancer of many factors like hereditary effects that are merge with environmental effects. There are, however, good reasons to think that the vast majority of cancers are initiated by genetic changes (mutations). The changes that lead to cancer are due to radiation, hormones, age factors etc. In a single cell, many changes (mutations) occur that helps them to divide in such a way so that it leads to cancer and it is transferred to the further generation [13].

In the current study, we have studied the Single nucleotide polymorphism in CYP1A1 gene. This gene is known to be a superfamily of enzymes which helps to catalyze many reactions i.e., cholesterol, steroids (estrogen), drugs. This gene is involved in cancer because it plays a role in the metabolism of polycyclic aromatic hydrocarbons and in the oxidative process of estrogens that helps to increase the risk of cancer [14].We will be finding out the occurrence and pattern of single nucleotide variant rs2645423 in the Himachal Pradesh Population to find out the susceptibility of this population towards lung cancer, cervical cancer and head and neck cancer due to the selected variant.

[2] REVIEW OF LITERATURE

To know about cancer, we have to know the definition of cancer. Cancer is a condition in which abnormal cell divide uncontrollable and can invade nearby tissue. It develops when a normal cell losesits control or stop working properly. As we know every cell in a human body have a life cycle. These cells have to proceed a sequence of phase, called cell cycle during which DNA duplicates [15]. Cell cycle have mainly four phases-

(1) G1 phase- This is also known as gap phase. In this phase cell grows larger and copies organelles.

(2) S- phase - This is also known as synthesis phase. In this phase cell synthesis, a complete copy of the DNA in its nucleus. It has also microtubule like structure called centrosome.

(3) G2 phase - It is also known as gap phase. In this phase cell grow, formation of protein and organelles take place and it is ready for mitosis. The G1, S and G2 phase together known as interphase.

(4) M-phase- The cell divide itself into two daughter DNA and cytoplasm to make new cells [16].

Cell cycle allows them to divide and produce new cells and after completion of cell cycle there is a senescence phase [17].Cell cycle is controlled by a series of signaling pathway by which cell grow and replicates DNA and divide itself into two daughter cells [18].This is the process by which errors are corrected and if not, the cells commit suicide (apoptosis) and cell death occurs. There are some regulatory pathways and checkpoints which allow the cells to respond quickly to damage DNA and also other form of stress which are required to cell cycle arrest and to prevent further uncontrolled division. Signaling mechanism play an impact on the control of cell cycle to allow cells to grow and divide in response to both development and environmental factors. Misregulation of cell cycle can lead to inappropriate cell cycle which leads to cancer. Sometimes there is a presence of mutation in the genetic material of cell which disturb the natural cell cycle. This leads to abnormal growth of the cell which leads to cancer [19].

Tumors are of two types-

Benign tumour: They are non-cancerous in the body unlike other tumour they don't propagate to different parts of the body but still they remain confined at one position. It can form lump or mass in the body. Even it can feel from outside the body [20]. It can be further classified on the site where it grows.

- Adenomas- Adenomas are structured like thin layer tissue which cover gland, organs etc and other structure in the body [21].
- Lipomas- These types of tumour exist from those cells which are fat and it is the most habitual type of benign tumour. It is mainly in the form of soft tissue.
- Myomas- It mainly grow from the muscle or in the walls of blood vessel [22].
- Fibroids- It also called as fibromas. It mainly grows on the fibrous or connective tissue of any organ.

Malignanttumour: They are cancerous in the body. It develops when the cells grow uncontrollable. Also, it will grow more rapidly and spread to different parts of the body. Some benign tumour can also convert into malignant tumour.[23]

TISSUE	MALIGNANT TUMORS	BENIGN TUMORS
Lymphoid Tissue	Hodgkin & Non- Hodgkin Lymphoma, Plasmacytoma, Multiple Myeloma	Plasmacytosis
Cells of Hematopoietic	Myeloproliferative disorders	Aleukemic Leukemia

Various malignant and benign tumours occurring in lymphoid and blood tissues.

[2.1] TYPES OF CANCER

The classification of cancer is based on their region where they occur in our body parts. They are mainly classified into 5 major groups-

- Carcinoma
- Sarcoma
- Melanoma
- Lymphoma
- Leukaemia.

Carcinoma: The cancerous cell mainly arises from cells that are epithelial i.e., it mainly begins from those tissue that lines the outer or inner surface of the body and that arise from those cells which are endodermal, mesodermal, ectodermal germ layer during embryogenesis [24]. Cells are further categorised into different category-

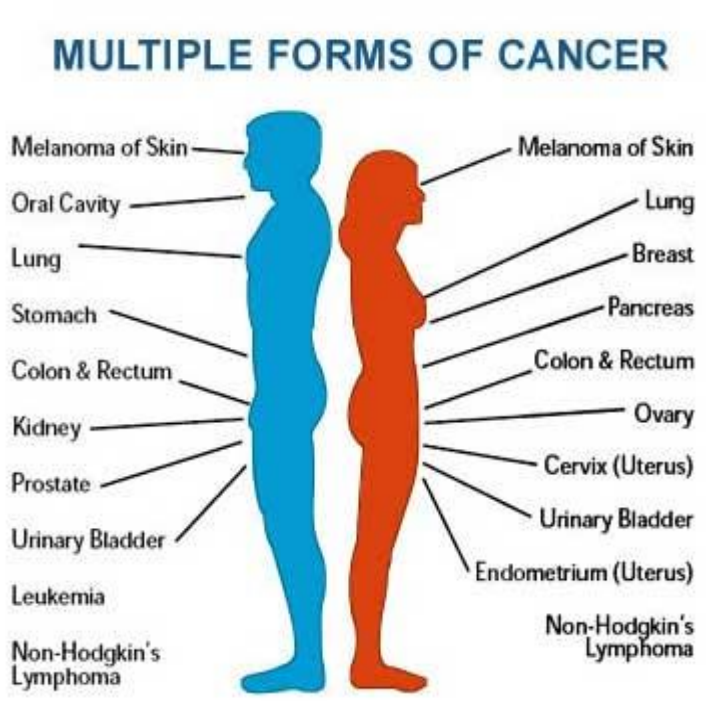
- Basal cell carcinoma: This is a type of epithelial cancer that is derived from rounded and small cells which are present in the epidermis (basal cells) and this is a form of skin cancer [25].
- Renal cell carcinoma: It is a form of kidney cancer. Also known as adenocarcinoma of renal cells or hypernephroma [26].
- Adenocarcinoma: It mainly begin in glandular cells that produces mucus like breasts, lungs, colon [27].
- Squamous cell carcinoma: This cancer is the 2nd most form of skin cancer. It is distinguished by unusual growth of cells (squamous) [28].
- Invasive ductal carcinoma: This cancer mainly began in the ducts of milk and spread to other parts of the body. Also called as Infiltrative ductal carcinoma [29].

Sarcoma: The cancerous cell mainly occurs in bones and in the soft connective tissue and also in soft tissue sarcoma [30]. They occur in bones, cartilage, ligaments, muscle, nerves, etc but they can also happen in others parts of the body [31].

Melanoma: It is a type of cancer that occurs in the skin cell that produces melanin-the pigment that gives skin colour [32].

Lymphoma: This is another type of cancer that is present in the lymphatic system which is a part of the infection -fighting cells of the immune system [33].These cells are in the lymph nodes, spleen, bone- marrow etc. they are further classified into two types i.e. non-Hodgkin and Hodgkin.

Leukaemia: It is a type of cancer that rise in the blood or bone marrow and then further spread to the other parts of the body by uncontrolled division and proliferation of abnormal cells. It is further categorised on the basis of how fast it develops i.e. acute leukaemia and chronic leukaemia [34].
On the basis of which type of cell is involved i.e. lymphocytic and myelogenous.



source: onlinesciencenotes.com/cancer-its-types-and-causes

CAUSES OF CANCER:

There are many cause and reason that lead to the development of cancer in the human body. Some of these are environmental factors, genetic factors, individual life- style, individual immunity and other risk factors. Cancer is caused by cancer causing substance and these cancer-causing substances known as carcinogenic and these carcinogenic substances lead to the development of cancer [35].

The risk factors of cancer may be further divided into sub- categories: -

- Biological factors- It is also known as body influenced factors or internal factors that include age, Gender, inherited genetic defects and skin type [36].

- Environmental factors can also be associated with the cancer, such as pollution, harmful gases anexposure to fine particulate matter environment [37].
- Occupational environment - It also influence cancer when a person is involving in carcinogenic. Substance in the form of aerosols, exposure to uv- radiation, harmful gases. some other factors also include poly-hydrocarbon factors and other metals compound have a risk associated with them.[38]
- Chemical or toxic compound exposure - benzene, nickel, cadmium, vine-chloride, asbestos, Nitrosamines, tobacco or cigarette.
- Pathogen: Human papilloma virus (HPV), Epstein-Barr virus, hepatitis virus B and C, Kaposi sarcoma associated herpes (KSHV), Schistosome species, Merkel cell polyomavirus helicobacter species these are the cancer-causing pathogen [39].
- Apart from these factors it can influence the way of lifestyle the way in which a person lives in his surrounding based on the level of hygiene, food and nutrition quality, particulate air in an aroundus [40].
- Approximately 20,000 genes are encoded in the human genome that are transcribed into 80,000 transcripts that are subsequently translated into proteins. These proteins alter the gene expression. Also, non- coding genome i.e. non-repetitive element cis- regulatory element. It also involved in modulating geneexpression.

Other risk factors associated with cancer is exposed to cancer causing substance like radiation it can be ionisable (X- ray) non-ionisable (UV radiation) these radiations might lead to cancer. Eating red meat such as (beef, lamb) also a high risk of cancer-causing agents. Cancer is a condition which arises in the person body remains in the pain during the whole treatment of cancer but it can also be developed by the excess use of certain drugs, or medicine that cause deficiency in the immune system.[41]

We have accomplished our study on the three types of cancer that are common in the population of Himachal Population-

- **Lung cancer**
- **Cervical cancer**
- **Head and neck cancer**

[2.2] Lung cancer

Lung cancer is also known as lung carcinoma. It is a type of malignant tumour that occur by uncontrollable cell growth in the lungs. Its growth can be spread beyond the lungs by the process of metastasis into nearby tissue or other parts of the body. It mainly occurs due to exposure to tobacco either active or passive [42].
There are two main type of lung cancer:

- Small cell lung carcinoma (SCLC) and Non-small cell lung carcinoma (NSCLC).

SCLC: It is also known as oat cell cancer. It is a type of malignant lung cancer found in the tissue of the lung and it cause 10-15% of all the lung cancer. Its growth rate is faster so it responds well to the chemo-therapy and radiation therapy.

NSCLC: About 80-85% lung cancer are non-small cell lung cancer and it is more lethal. It can be categorised on the basis of carcinoma like adenocarcinoma, squamous cell carcinoma and large cell carcinoma.

Figure 3- Lung Cancer
Source: medicaldialogues.in

CAUSES:

There are numerous risk factors for the cause of cancer some may be environmental, genetics, life-style, etc and the risk factors that can be associated with the chance of occurrence of carcinoma in the body. Some of the factors may be include-

1. **Smoking**: It is said to be the main causes of lung cancer. It is linked to 80-90% of lung cancer death. It affects both the people either you have done active smoking or passive smoking. It mainly leads to the production of carcinogen in your body that will lead to the formation of lung cancer. It also depends on which form of tobacco you smoked and for how long. Cigarette is mainly a cause of cancer of mouth, throat and oesophagusetc [43].
2. **Cigar smoking and pipe smoking:** It is also a cause of lung cancer. Smoking with low tar or light cigarette can also increase the risk of cancer. Methanol cigarette smoking can cause more risk because it inhales more deeply inside the body [44].
3. **Exposure to radon**: It is a radioactive gas that is form from the breakdown of uranium in the soil and rock. It is colourless and tasteless. It is the second most leading cause of cancer and it mainly affect non- smoker. It's exposure to small amount of radiation affect the lung [45].
4. **Exposure to asbestos and other carcinogen**: People who work with these asbestos (such as mine, textile plants) are having more chance of lung cancer because they exposed to large amount of asbestos which have a high risk of developing mesothelial (a type of pleural cancer) [46].
5. **Family history**: The chances of lung cancer is much more if your parents/relative are already having a history of lung cancer.

SYMPTOMS:

The symptoms of lung cancer are basically same for both small cell lung cancer (SCLS) and non-small cell lung cancer (NSCLS).

- Coughing up phlegm or blood
- Breath Shortness
- Chest pain
- Lingering or worsting cough
- Loss of appetite and weight

STAGING IN LUNG CANCER:

It tells us how far cancer has been spread out in your body and it also guide the doctor for the treatment. The chances of curative treatment are successful when it is diagnosed in early stage or before it spread but it doesn't cause obvious symptoms in early stage or before it spread. NSCLC has mainly 4 stage:

STAGE 1: When the cancer is located only in the lungs but it doesn't proliferate outside the lungs.
STAGE 2: When the cancer is found in the lungs, further it spread to neighbouring lymph node.

STAGE 3: When the cancer is present in the lungs, lymph node & in the middle of chest.

STAGE 3A: It extend to the lymph node on the same side of the chest from where the cancer has been start growing.

STAGE 3B: It extends to the lymph node on the opposite side of the chest.

STAGE 4: When the cancer has been spread to both the lungs, into an area around the lungs and distant organ also.

TREATMENT:

Depending upon the stage of cancer these possible treatments are preferred-

- Radiotherapy- It uses beam of intense energy to kill cancerous cell. Most often x- raypreferred.
- Chemo therapy- It has been widely performed in clinic because of its simple and convenient process. It is a chemical form of drug therapy that is meant for destroy rapidly growing cells in the body.
- Surgery- It employ operation or treatment of disease. It involves cutting, suturing or physically changing body tissue or anotherorgan.
- Targeted therapy- antibodies or antibody -drug conjugates targeting cell receptor are used to destroy rapidly growing cell. It utilizing surface receptor cell which are highly expressed on tumourcell.[47]

PREVENTION:

Avoid smoking: Tobacco smoking is the common cause of cancer. The disease goes into metastasis and it spread to different parts of the body. Meanwhile, a person who has not smoke ever then the chance of cancer reduces [48].

Healthy diet: The way to avoid any disorder is to take healthy and nutritious food help in maintaining the body's immunity. Intake of vitamin can help in reducing harmful carcinogenic substances [49].

Avoid carcinogenic: Proper steps and measures should be taken while working the environment where carcinogenic effects are prominent. Also, they have to follow safety measures that are guided by the institution of chemical factory while working.

Exercise: The best way to stay healthy can be achieved by doing physical exercise. These help in detoxifying carcinogenic substance from the body and produces good immunity [50].

[2.3] HEAD AND NECK CANCER

It includes a number of malignant tumours that born in the throat, lips, larynx, nose, sinus etc. It is mainly squamous cell carcinoma. It mainly began in the squamous cells (flat) that helps to make up layer of tissue (thin) on surface of structure in the neck and head. When a cancer is found in layer of squamous then it is said to be carcinoma (in situ) or when it moved into tissue than it is known as invasive squamous cell carcinoma.[51]

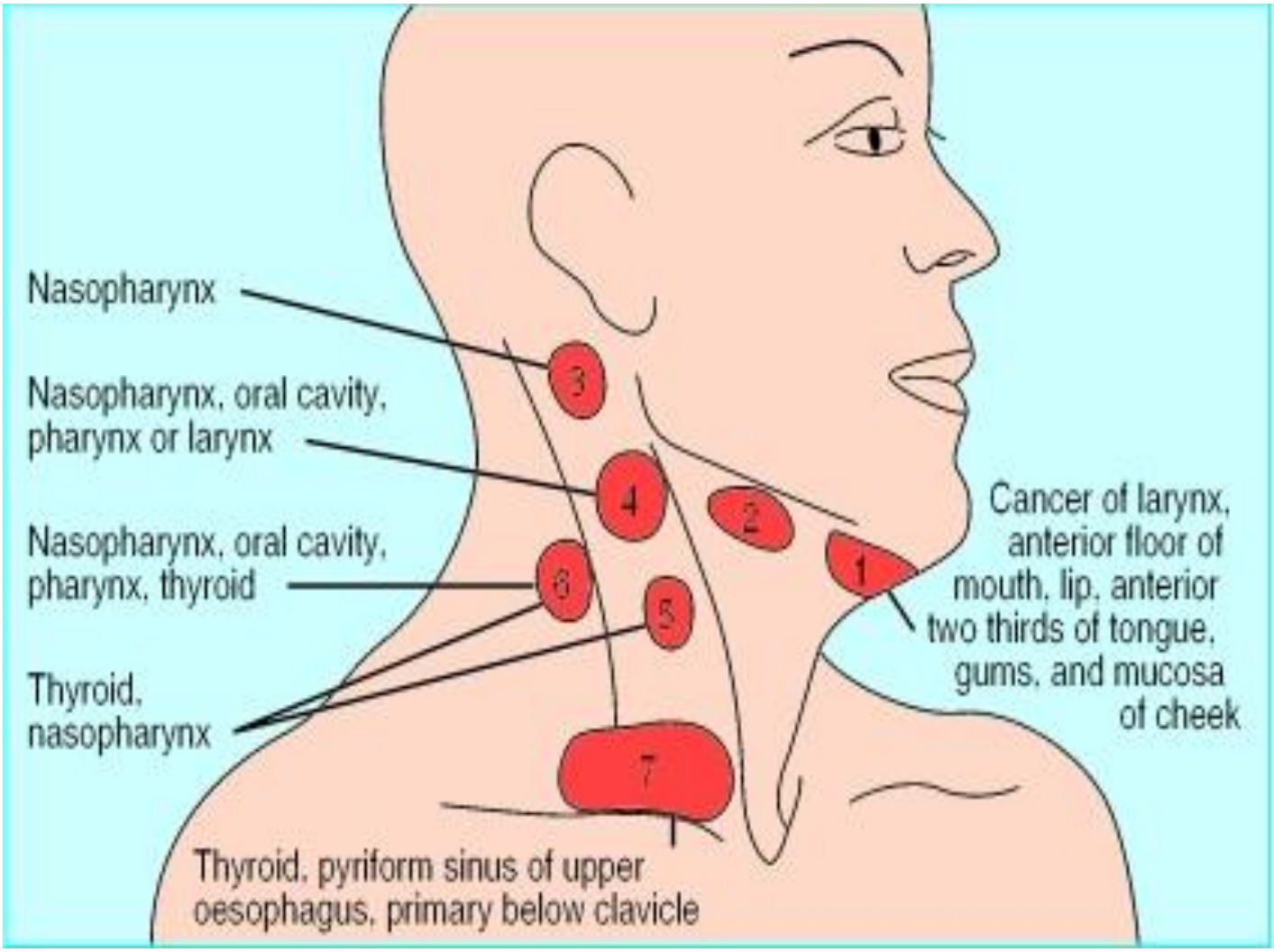


Figure4: Anatomy of a region showing head and neck cancer

Source :/mdbcancerjourney.com

TYPES:

They are of mainly 5 types of head and neck cancer and each one of them are named according to the body part.

Laryngeal and hypo-pharyngeal cancer- It mainly grow in the lower part of the throat.

Laryngeal cancer it mainly affects the voice box which contain vocal cords. Whereas hypo pharynx is the part of the throat that lie behind the larynx. It is mainly associated with the thin flat cell of squamous cell carcinoma [52].

Oral and oropharyngeal cancer- oral cancer is also known as oral cavity cancer. It mainly starts in the mouth.

It also covers lip, cheeks, gums, teeth etc. Whereas oropharyngeal cancer mainly starts in the oropharynx. This part is present in the throat just behind the mouth. This is mainly associated with the squamous cell carcinoma [53].

Nasal cavity and paranasal sinus cancer- It is a tumour that grow inside your sinuses, the space around the nose where mucus is produced, or the space where air passes into the lungs.

It is a disease in which malignant cancer cells form in the tissue [54].

Salivary gland cancer- It is rare malignant cancerous cell form in the mouth of the tissue.

Salivary gland makes saliva and release into the mouth.

There are three major salivary glands on which parotid's gland is largest one and is most likely cause malignant

Cancerous cell [55].

Nasopharyngeal cancer -It is a type of throat cancer that develop when cancerous cell grow in the nasopharynx

the upper part of the throat. This disease doesn't cause any symptoms in the early stage of the cancer often it

might develop in the latter stage. It is also called as nasopharyngeal carcinoma and it is mainly associated with pharyngeal mucosal space [56].

CAUSES AND RISK FACTORS:

Some factors that can influence cancer caus. Excess intake of carcinogenic product lead to the development of

benign and malignant types tumour. Some risk factors are-

Alcohol: Frequent and heavy consumption of alcohol can increase the risk of developing cancer in the mouth, pharynx and larynx.[57]

Tobacco: It is the main leading cause of cancer. Chewing tobacco and its snuff is the main reason of this cancer.[58]

Sun exposure: It is mainly linked to the cancer in the lip as well as in the skin area.[59]

Epstein Barr virus: This virus cause mononucleosis or mono that plays a role in nano pharyngeal disorder.[60]

Age: People who are the age at above 40 have high risk of HNC.

Poor Diet: A diet having low in vitamin A and B cause high risk of HNC.

Ancestry: Hereditary factors also plays an important role in cancer.

DETECTION:

For examine any disease first we have to diagnosed a disease.

CT of sinuses

CT scan

Panoramic dental X-ray

Endoscopy

Biopsy

PET CT scan

Bone scan

TREATMENT:

Depending upon the size, location of the cancer and its growth rate. These treatments were recommended-

Chemotherapy

Radiation therapy

Surgery

Targeted therapy

Immunotherapy

PREVENTION:

1. Stop intaking tobacco products [61].
2. Avoiding drinking alcohol.
3. Using sunscreen: It contains sun protection factor that reduce the chance of cancer [62].
4. Maintain good care of denatures: Poor denatures can catch tobacco and alcohol [63].

Regular appointment at dentist can help in avoiding formation of unwanted plaque.

[2.4] CERVICAL CANCER

It happens when there is change in the cell present in women cervix. It connects uterus to the vagina. Cancer affect the deeper cervix tissue and it will spread to the different parts of the human body. It may be caused by the infection with human papillomavirus. The growth rate of cancerous cell is slow in cervical cancer so there is enough time to detect and treat the disease before it causes serious problems.[64]

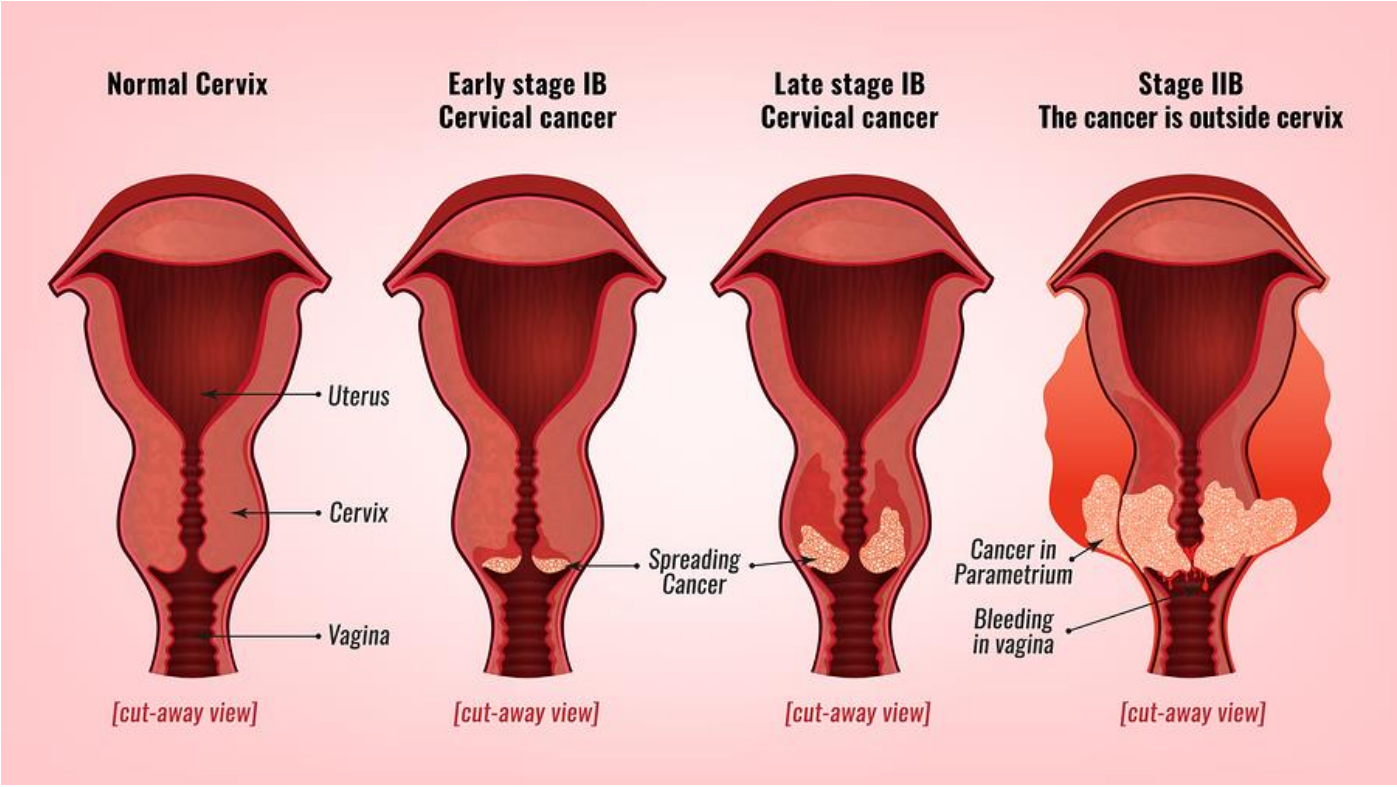


FIGURE 5- Cervical Cancer (This figure shows different stages of cervical cancer)

Source- https://hmcisrael.com/

It can be classified on the type of cell that will lead to form in the cervical cancer i.e. squamous cell carcinoma and adenocarcinoma.

- **Squamous cell carcinoma**- It mainly begins in the thin flat cells that line the outer part of the cervix. It mainly projects towards the vagina. Most common type of cervical cancer.
- **Adenocarcinoma**- It mainly begins in the column- shaped glandular cells which line the cervical canal. It is a rare type of cervical cancer.

Cause and risk factors:

There are several factors that increase the cause of cervical cancer.

- **Many sexual partners**- As the more sexual partner more the chance of cause of cancer. The transmission of cancer-causing HPV virus mainly occurs through sexual contact with an individual who has HPV.[65]
- **Early sexual activity**- sex at any early stage increase the chance of human papilloma virus any they cause cancer.

- **Smoking -** It is the cause of cancer. It is mainly associated with the squamous cell cervical cancer.[66]
- **A weakened immune system**- It will be more likely to develop cancer as the immune system is weakened. As the weekend immune system, the risk of cervical cancer is higher in those people have infected with HIV, AIDS or who have undergone transplant.
- **Birth control pills**- Exposure to certain drugs like diethylstilboestrol (DES) while pregnant, or consumption of contraceptive pills rise the cause of cervical cancer.
- **Other sexually transmitted disease**- it will increase the cause of cervical cancer e.g. chlamydia, gonorrhoea and syphilis.

DIAGNOSIS OF CERVICAL CANCER:

- ➢ Pap test
- ➢ Pelvic exam
- ➢ Colposcopy
- ➢ Biopsies
- ➢ CT scan
- ➢ MRI

STAGING OF CERVICAL CANCER:

It refers to the extends to which it has been spread in the body at the time of diagnose. On basis of cancer stage treatment preferred.

In general, there are five stages:

1. STAGE 0: The cells that are abnormal are only on the surface of cervix.
2. STAGE 1: A very little amount of tumours is present but it will not been spread in others parts of body.
3. STAGE 2: The tumour has been extended to the cervix and also uterus but doesn't occupy any wall of pelvic or vagina
4. STAGE 3: In this cancer has extended into the lower part of vagina or on wall of pelvic. Moreover, it starts blocking of uterus.
5. STAGE 4: This is the advanced stage of cancer, now the cancer has spread to the bladder or to the rectum, any other part of body.

TREATMENT:

After detection of disease it is necessary to take possible treatment and option. Some of the treatments are:

- Chemo therapy -It has been widely performed in clinic because of its simple and convenient process. It is a chemical form of drug therapy that is meant for destroy rapidly growing cells in the body.

- Hysterectomy- A surgical process in which you remove your uterus through an incision in your low-erabdomen.
- Surgery-It employ operation or treatment of disease. It involves cutting, suturing or physically changing body tissue or anotherorgan.
- Biologicaltherapy- It use living organism or substance derived from living tissue to treatdisease.
- Radiationtherapy -It uses beam of intense energy to kill cancerous cell. Most often x- raypreferred.

PREVENTION:

To reduce the chance of cervical cancer following steps should be taken:

1. HPV vaccine: HPV is the main cause of cervical disorder (cancer) which occurs in the body of women. So, to avoid the cause of infection and reduce the risk of cervical cancer HPV vaccination should be taken.[67]
2. Pap test: It can find out the precancerous order of the cervix.
3. Safe- sex: It help in preventing from sexually transmitted disease. Use of condoms and other physical contraceptive barriers.
4. Few sex partners: It reduces the chance of getting HPV and its type and also it reduces the chance of getting cancer.

[2.5] CYP1A1

The CYP1A1 gene (cytochrome p450) encodes for those enzymes that belongs to the super family of cytochrome P-450. Those enzymes which are belonging to cytochrome P450 super family are known to be mono-oxygenases that catalyse those responses which are numerous and involved with cholesterol group, steroids and many kinds of lipids.[68] These are present in very little amount in the endoplasmic reticulum. It may process few PAHs to tumour that causes intermediates.[69] It is mainly associated with the increase risk of lung cancer. As it has a major role involved in the metabolism of polycyclic hydrocarbons (aromatic) and also in the metabolism (oxidative) of some hormones like estrogen that increases the chances of cancer and oxidative stress. The variations that occur in the CYP1A1 gene is linked with the increased activity of aryl hydrocarbon hydroxylase and also effect person's susceptibility to cancer [70].

It catalyses the conversion of polycyclic aromatic hydrocarbon into reactive metabolites which may participate in the initiation of carcinogen via the formation of bulky PAH -DNA adducts. The PAH- DNA adduct is considered as a molecular marker. Gene expression can be altered by epigenetic change such as DNA methylation and modification of histone protein. CYP1A1 gene control transcriptional region. It is xenobiotic metabolizing enzyme involved in the metabolizing of number of toxins, drug and endogenous hormone. Polymorphism in the gene can alter enzyme activity and it is also associated with the susceptibility of cancer, environmental toxin and hormone exposure [71].

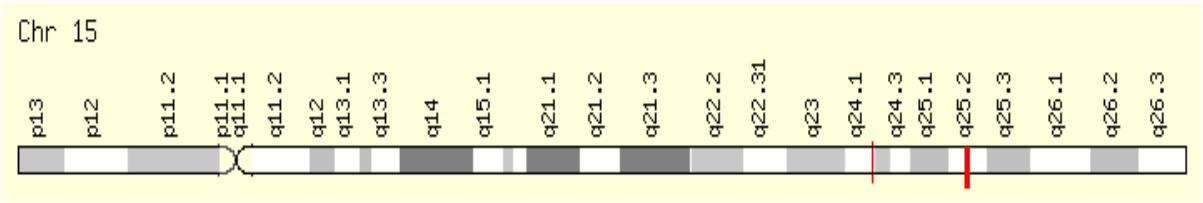


Figure 6- Genomic location of CYP1A1 gene (Highighted in red)

Source: Genecards

[2.6] SINGLE NUCLEOTIDE POLYMORPHISM

SNP is abbreviated as single nucleotide polymorphism which is the most common kind of genetic variation in the people. It occurs almost 1 in every 1000 nucleotides. Each SNP represent a difference in a single nucleotide. These single nucleotides also known as a DNA building block. SNP may replace nucleotide (cytosine) with the nucleotide thymine [72].

It means that the population more than 1% does not have the same type of nucleotide in the DNA sequence at specific position, then this type of change is said to be SNP. It represents a genetic marker to understand complex disease [73]. When it occur in the coding region, it is more likely to alter protein product this will lead to change phenotype, such as classical monogenic disorder (sickle cell disease or haemophilia). Also, the SNP that are involved in a gene then this type of gene is known to have more (than one) allele If SNP occurs within a gene then the gene is described as having more than one allele. This (SNP) is leading to changes in the sequence of amino acids [74].

The SNP is said to be in non-coding region of gene, coding region, intergenic- region. SNP that occurs in coding sequence may have the capability to change the sequence of amino acid due to some reasons like degeneracy [75]. The SNP the are present in the coding region that are of 2 types-

Non-synonymous: It helps to change the sequence of amino acids.

Synonymous: It does not at all change or affect the sequence of protein.

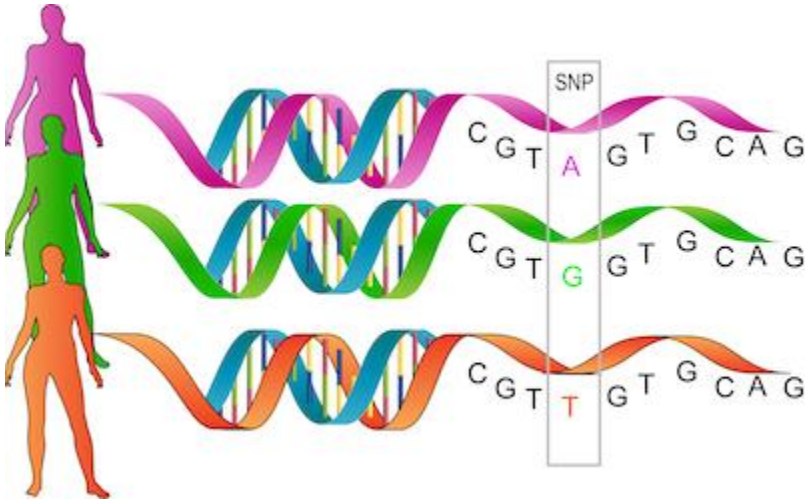


Figure 7- Single Nucleotide Polymorphism are DNA differences at a particular location.

Source-https://genetics.thetech.org

This SNP is present on chromosome number 15, C— G.[76]

These are some research paper that we have studied to know the effect this SNP is this is a cause of disease or not.

Case study - It was conducted on polymorphism of osteoporosis in postmenopausal women in IranPopulation. A total 112 samples were taken for analyzing. we analyze that mutant haplotype was at greater risk of osteoporosis. [77]

Case study- It was conducted on hepatitis B virus on Iranian population. A total 54 samples were taken for analyzing. We analyze that TT genotype is more susceptible to hepatitis B virus.[78]

Case study-It was conducted to associate the relationship between paternal smoking and childhood Leukemia on Korean population. A total 164 samples were taken for analyzing. We analyze that Paternal Smoking increase the risk of childhood leukemia.[79]

Case study- It was conducted on bladder cancer associated with coffee consumption on Spanish Population.A total 1136 samples were taken for analyzing. We analyze that bladder cancer risk was more prominent among coffee drinkers.[80]

Case study-It was conducted on hepactocelluer carcinoma on Chinese population. A total 1006 sampleswere taken for analyzing. We analyze TTGC diplotype increased risk of hepatocellular carcinoma.[81]

Case study-It was conducted on lung cancer associated with quercetin- rich food intake on the Eagle Population (Lombardy region of Italy). A total 2100 samples were taken for analyzing. We analyze that intake of quercetin rich product lowers the risk of lung cancer in Italian population.[82]

The main aim of this study is to find out the occurrence of SNP rs2198843 in CYP1A1 gene to find out its role susceptibility of Himachal Pradesh Population towards common cancers.

[2.7] PCR-RFLP

What basically RFLP is- It is known to be an enzyme i.e. restriction endonuclease that helps to cut the deoxyribonucleic Acid (DNA) at specific sites. Restriction Fragment Length Polymorphism is a (technique) method which is used to observe (find) the dissimilarity in a sequence of DNA that is known as polymorphism. The transforming patterns are used to observe the difference among variant organism and these types of transforming patterns are known as VNTRs (Variable Number of Tandem Repeats).[83]

In this study, we are using RFLP to find the susceptibility of the gene CYP1A1 in the Himachal Pradesh Population with different types of cancer like Lung cancer, Cervical Cancer, Head and Neck Cancer.Restriction Fragment Length Polymorphism is a method (technique) which is used to observe (find) the dissimilarity in a sequence of DNA that is known as polymorphism. The transforming patterns are used to observe the difference among variant organism and these types of transforming patterns are known as VNTRs (Variable Number of Tandem Repeats).

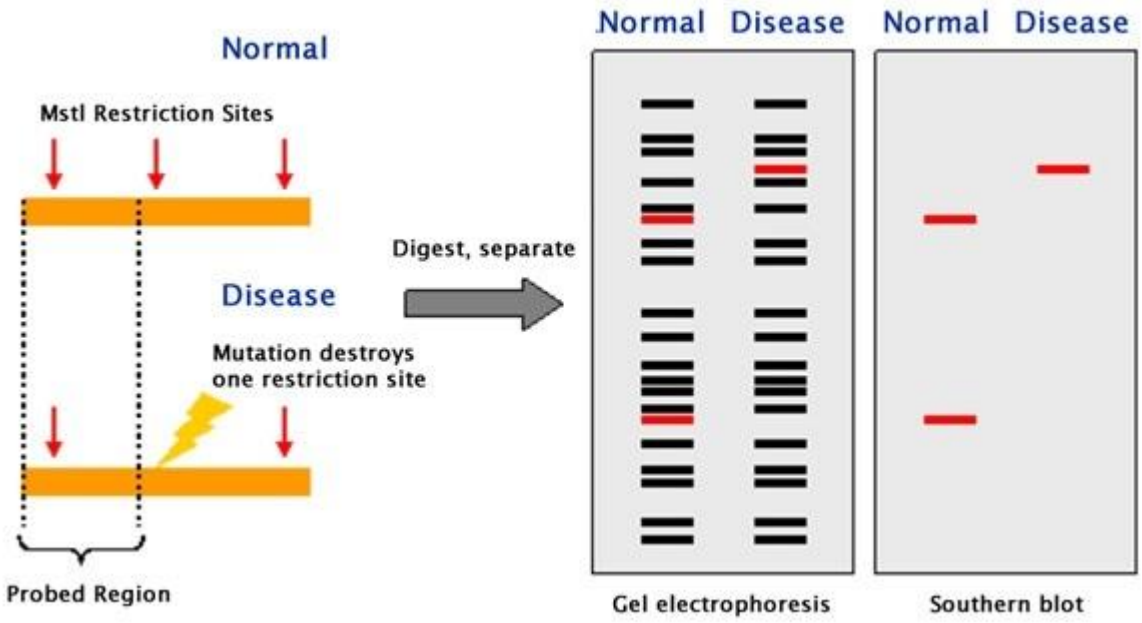


FIGURE 8 - RFLP (Restriction Fragment Length Polymorphism)

Source- https://www.news-medical.net

[3] Objectives

Following are the 2 main objectives of this study:

- First step is the PCR-RFLP optimization set-up for the SNP rs2198843 by using the technique of gradient PCR having multiple ranges of temperature.
- Next step is the analysis of the relation of the SNP rs2198843 with CYP1A1 gene and to know its susceptibility with the cancer in the Himachal Pradesh population.

[4] MATERIALS AND METHODS

Our study is basically on the Himachal Pradesh population. We have a total of 395 samples of the cancer and about 212 samples of control. The samples were collected according to the age and geographical distribution of HP Population. The samples were taken after the consent of the patients. For this study, the isolation of DNA was performed.

SAMPLING:

The sampling was done under the proper supervision and guidelines of the doctor and the patient. Approximate 5ml of blood was drawn from each patient with the help of proper sterilized needles and then it was stored in coated vial of EDTA. Then it was placed inside the refrigerator to avoid degradation of DNA and clotting of blood sample at -20 degree Celsius.

ISOLATION OF GENOMIC DNA:

The isolation of DNA from blood was done by the method of Salting Out method and it was stored in separate vial at a temperature -20^{o}C. These are the following steps for the isolation of DNA by salting out method

- 300 micro-litres of blood sample + 900 micro-litre RBC lysis buffer (3x of blood sample) and kept on Rocker for incubation at room temperature for the lysis of RBC.
- To obtain white pellet of WBC centrifugation was done at 13000rpm.
- Discard supernatant, WBC pellet was suspended in 300 micro-litre TE buffers at pH 8.0. Add 20 micro-litres of 10% SDS buffer and placed on dry bath for incubation at 56 degree Celsius.
- Add 150 micro-litre of 7.5M ammonium acetate and mixed vigorously for 1minute. Centrifugation was done at 13000rpm for 15min which will separate the protein as well as precipitate.
- Clear supernatant was transferred into fresh micro- tube. Add chilled ethanol (twice the volume of Supernatant). It will precipitate the genomic DNA.
- Precipitated genomic DNA were centrifuged at 13000rpm for 10 min, to pellet at the bottom of the micro-tube. Subsequently washed the pellet DNA with 70% ethanol and air dried at room temp for 10-15 min.
- 100 micro-litres of TE buffer pH 7.3 was added to dissolve the DNA and it was stored at -20 till further use.
- Run the DNA on 0.8% agarose gel using Ethidium Bromide.
- DNA band were observed.

In this study, we got the primers from the study of reference (using BLAST) in which our entire study depends.

RECONSTITUTION OF PRIMERS:

The primers that were obtained we made different dilutions and also reconstituted it. Control Samples were used for the same. And concentrations were also made and optimized up to 100uM. Nuclease free H_2O was also added to the primers that were lyophilized.

PRIMERS	NUCLEASE FREE WATER (uL)
rs2198843 Forward	450
rs2198843 Reverse	450

PCR OPTIMIZATION:

Now, for the optimization process, Gradient (PCR) was run by using different temperature range. Through this the best annealing temperature was obtained.

REACTION COMPONENTS	PER REACTION VOLUME (ul)
Nuclease Free H_2O	5.6Ul
DNA Template	1Ul
Annealing Temperature	58, 59, 60, 61, 62
Primer (Forward)	.2uL

Primer (Reverse)	.2uL
Master Mix	5uL
Overall	12uL

TABLE:

Cycle of PCR for rs2198843

STEPS	TEMPERATURE (^{0}C)	TIME	NO. OF CYCLES
Denaturation (Initial)	94.0	4.0 minutes	1.0
Denaturation	94.0	35 second	35.0
Annealing	60.0	35 second	35.0
Extension	72.0	1 minute	35.0
Extension (Final)	72.0	5 minutes	1.0
Hold	4	A	-

GENOTYPING BY RFLP:

When the optimization was done and suitable annealing temperature was obtained (optimized) at 60^0C, then further PCR was run. After the whole process genotyping was made. Then the result was seen (visualized) on 1.2% agarose gel. Also, a dye was also used i.e., Ethidium Bromide (EtBr).

Pst1 enzyme was also used for restriction digestion. After the process it was incubated at 37^0C for 11-12 hours.

ANALYZATION OF PRODUCT THAT IS DIGESTED:

When the product of PCR was taken under the digestion by using Pst1, the products that were obtained at last were seen on agarose gel. Through Gel dock with the help of UV the banding pattern were observed.

TABLE: Following are the different reaction components and volume for restriction digestion

COMPONENTS (REACTION)	**VOLUME (REACTION) (ul) for one reaction**
Restriction enzyme	0.2
PCR amplified	10.0
Nuclease Free H_2O	3.50
Buffer	1.50
Overall	15.0

TABLE: Following are the different genotypes with banding patterns

GENETIC CONSTITUTION	**SIZE OF BAND (bp)**
Wild Type (GG)	404
Heterozygous (GC)	404+299+105
Mutant Homozygous (CC)	299+105

[5] RESULTS

1.

L B 58^0C 59^0C 60^0C 61^0C 62^0C

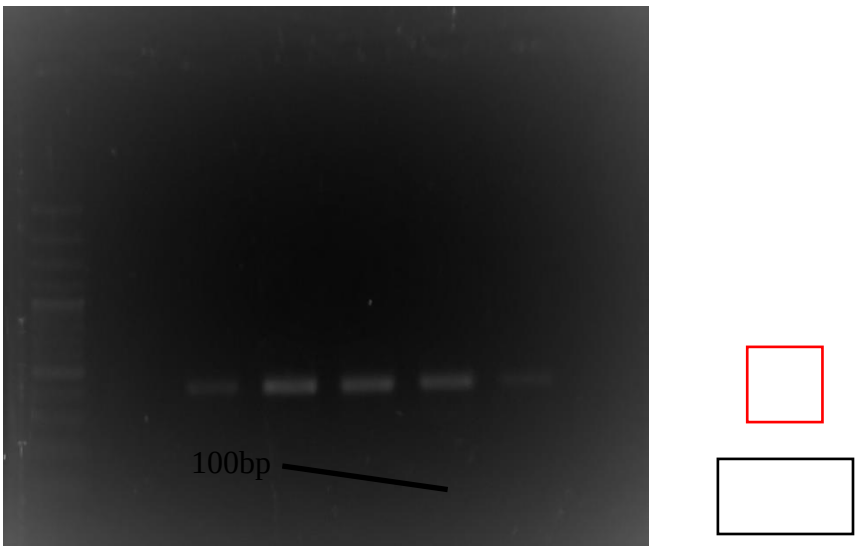


Fig. 1- Gradient PCR check gel

2. L -ve 1 2 3 4 5 6

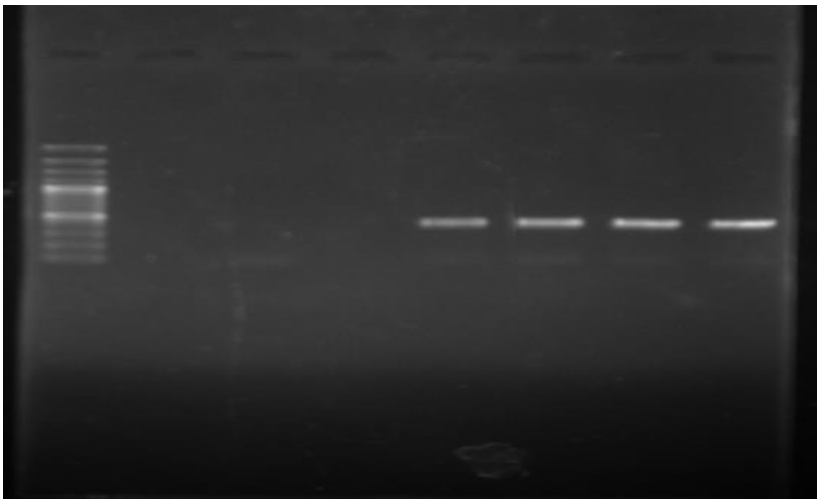


Fig. 2- PCR Check Gel

3.

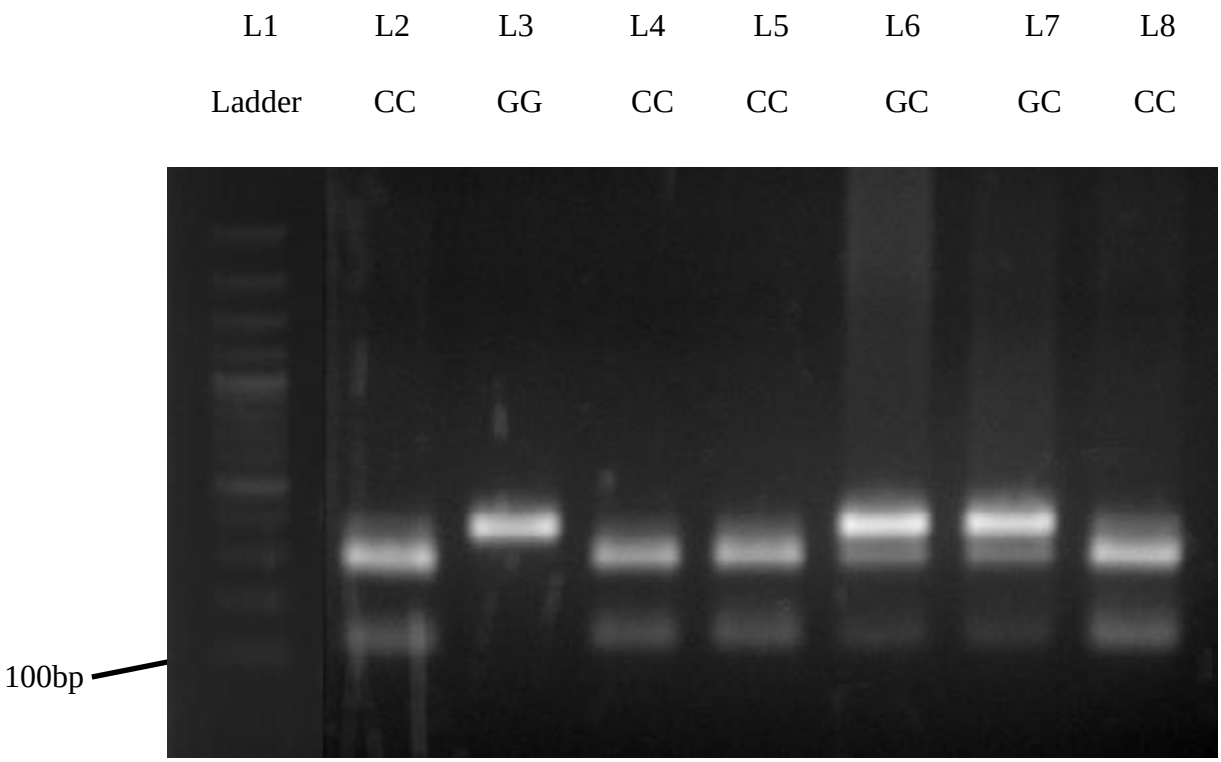


Fig. 3- Restriction Digestion Gel

Restricted Fragments Size

GG= 404bp

GC= 404bp + 299bp + 105bp

CC= 299bp + 105bp

Due to lockdown, we were unable to analyze the results. So, when the college re opens we will analyze our results.

REFERENCES

[1] A. Knudson, "Genetics of Human Cancer", *Annual Review of Genetics*, vol. 20, no. 1, pp. 231-251, 1986.

[2] J. Prensner and A. Chinnaiyan, "The Emergence of lncRNAs in Cancer Biology", *Cancer Discovery*, vol. 1, no. 5, pp. 391-407, 2011.

[3] A. Knudson, "Genetics of Human Cancer", *Annual Review of Genetics*, vol. 20, no. 1, pp. 231-251, 1986.

[4] E. Knudsen, R. Nambiar, S. Rosario, D. Smiraglia, D. Goodrich and A. Witkiewicz, "Pan-cancer molecular analysis of the RB tumor suppressor pathway", 2020.

[5] R. Lavker and T. Sun, "Epidermal stem cells: Properties, markers, and location", 2020.

[6] Z. Lima, M. Ghadamzadeh, F. Arashloo, G. Amjad, M. Ebadi and L. Younesi, "Recent advances of therapeutic targets based on the molecular signature in breast cancer: genetic mutations and implications for current treatment paradigms", *Journal of Hematology & Oncology*, vol. 12, no. 1, 2019.

[7] R. Bollmann, "J. J. Bullen and E. Griffiths (Editors), Iron and Infection — Molecular, Physiological and Clinical Aspects. IX + 325 S., 70 Abb., 30 Tab. Chichester-New York-Brisbane-Toronto-Singapore 1987. John Wiley and Sons, £ 38.00. ISBN: 0-471-91168-2", *Journal of Basic Microbiology*, vol. 27, no. 8, pp. 448-448, 1987.

[8] D. Bleyl, "IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Genetic and Related Effects: an updating of selected IARC Monographs from Volumes 1 to 42. Supplement 6. 729 Seiten. World Health Organization/International Agency for Research on Cancer 1987. Preis: 80, –- Sw.fr.", *Food / Nahrung*, vol. 33, no. 10, pp. 956-956, 1989.

[9] P. Sugerman, B. Joseph and N. Savage, "The role of oncogenes, tumour suppressor genes and growth factors in oral squamous cell carcinoma: a case of apoptosis versus proliferation", *Oral Diseases*, vol. 1, no. 3, pp. 172-188, 2008.

[10] J. Butel, "Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease", *Carcinogenesis*, vol. 21, no. 3, pp. 405-426, 2000.

[11] R. Doll and R. Peto, "The Causes of Cancer: Quantitative Estimates of Avoidable Risks of Cancer in the United States Today", *JNCI: Journal of the National Cancer Institute*, vol. 66, no. 6, pp. 1192-1308, 1981.

[12] A. Feinberg and B. Tycko, "The history of cancer epigenetics", *Nature Reviews Cancer*, vol. 4, no. 2, pp. 143-153, 2004.

[13] B. Fox and J. Goldsmith, "Behavioral issues in prevention of cancer", *Preventive Medicine*, vol. 5, no. 1, pp. 106-121, 1976. Available: 10.1016/0091-7435(76)90014-1.

[14] P. Peltomaki and H. Vasen, "Mutations predisposing to hereditary nonpolyposis colorectal cancer: Database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer", *Gastroenterology*, vol. 113, no. 4, pp. 1146-1158, 1997.

[15] M. Nordstrom-O'Brien et al., "Genetic analysis of von Hippel-Lindau disease", *Human Mutation*, p. n/a-n/a, 2010.

[16] L. Agrawal, X. Lu, Q. Jin and G. Alkhatib, "Anti-HIV Therapy: Current and Future Directions", *Current Pharmaceutical Design*, vol. 12, no. 16, pp. 2031-2055, 2006

[17] B. P.B. Danielson, "The Cytochrome P450 Superfamily: Biochemistry, Evolution and Drug Metabolism in Humans", *Current Drug Metabolism*, vol. 3, no. 6, pp. 561-597, 2002.

[18] N. LESLIE and C. DOWNES, "PTEN function: how normal cells control it and tumour cells lose it", 2020.

[19] K. Herrup and Y. Yang, "Cell cycle regulation in the postmitotic neuron: oxymoron or new biology?", *Semanticscholar.org*, 2020.

[20] J. Massagué, "G1 cell-cycle control and cancer", 2020.

[21] C. Shaha, R. Tripathi and D. Mishra, "Male germ cell apoptosis: regulation and biology", 2020.

[22] D. Bleyl, "IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Genetic and Related Effects: an up dating of selected IARC Monographs from Volumes 1 to 42. Supplement 6. 729 Seiten. World Health Organization/International Agency for Research on Cancer 1987. Preis: 80,–- Sw.fr.", *Food / Nahrung*, vol. 33, no. 10, pp. 956-956, 1989.

[23] L. Agrawal, X. Lu, Q. Jin and G. Alkhatib, "Anti-HIV Therapy: Current and Future Directions", *Current Pharmaceutical Design*, vol. 12, no. 16, pp. 2031-2055, 2006.

[24] J. Kaude, C. Silseth and U. Tylen, "Angiography in myomas of the gastrointestinal tract.", *UC Davis*, 2020.

[25] "Resveratrol Protects Chondrocytes from Apoptosis - MAFIADOC.COM", *mafiadoc.com*, 2020.

[26] R. Keller, "An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation ofXenopuslaevis", 2020.

[27] A. Crowson, "Basal cell carcinoma: biology, morphology and clinical implications", 2020.

[28] M. ANDERSON and D. ATKINSON, "Renal Carcinoma in Pregnancy", 2020.

[29] K. Motaparthi, J. Kapil and E. Velazquez, "Cutaneous Squamous Cell Carcinoma", 2020.

[30] L. Agrawal, X. Lu, Q. Jin and G. Alkhatib, "Anti-HIV Therapy: Current and Future Directions", *Current Pharmaceutical Design*, vol. 12, no. 16, pp. 2031-2055, 2006.

[31] C. Shaha, R. Tripathi and D. Mishra, "Male germ cell apoptosis: regulation and biology", 2020.

[32] R. Bollmann, "J. J. Bullen and E. Griffiths (Editors), Iron and Infection — Molecular, Physiological and Clinical Aspects. IX + 325 S., 70 Abb., 30 Tab. Chichester-New York-Brisbane-Toronto-Singapore 1987. John Wiley and Sons, £ 38.00. ISBN: 0-471-91168-2", *Journal of Basic Microbiology*, vol. 27, no. 8, pp. 448-448, 1987.

[33] D. Gabrilovich and S. Nagaraj, "Myeloid-derived suppressor cells as regulators of the immune system", 2020.

[34] Y. Shiozawa et al., "GAS6/AXL Axis Regulates Prostate Cancer Invasion, Proliferation, and Survival in the Bone Marrow Niche", 2020.

[35] M. Clemens, "Free radicals in chemical carcinogenesis", 2020.

[36] J. Berek et al., "Molecular and biological factors in the pathogenesis of ovarian cancer", *Annals of Oncology*, vol. 4, pp. S3-S16, 1993.

[37] R. Montesano and J. Hall, "Environmental causes of human cancers", *European Journal of Cancer*, vol. 37, pp. 67-87, 2001.

[38]R. Clapp, M. Jacobs and E. Loechler, "Environmental and Occupational Causes of Cancer: New Evidence 2005-2007", *Reviews on Environmental Health*, vol. 23, no. 1, pp. 1-38, 2008.

[39]J. Parsonnet, "Bacterial infection as a cause of cancer.", *Environmental Health Perspectives*, vol. 103, no. 8, pp. 263-268, 1995.

[40] B. Ames, L. Gold and W. Willett, "The causes and prevention of cancer.", *Proceedings of the National Academy of Sciences*, vol. 92, no. 12, pp. 5258-5265, 1995.

[41]H. Ananthaswamy, "Mechanisms of induction of skin cancer by UV radiation", *Frontiers in Bioscience*, vol. 2, no. 4, pp. d538-551, 1997.

[42]A. Alberg and J. Samet, "Epidemiology of Lung Cancer*", *Chest*, vol. 123, no. 1, pp. 21S-49S, 2003.

[43] K. Jung, C. Jeon and S. Jee, "Smoking effect on lung cancer: ethnic difference and smoking paradox", *Epidemiology and Health*, p. e2016060, 2016.

[44] A. Alberg and J. Samet, "Epidemiology of Lung Cancer*", *Chest*, vol. 123, no. 1, pp. 21S-49S, 2003.

[45]"Lung cancer in radon-exposed miners and estimation of risk from indoor exposure", *Lung Cancer*, vol. 13, no. 2, p. 188, 1995.

[46]B. Mossman, D. Kamp and S. Weitzman, "Mechanisms of Carcinogenesis and Clinical Features of Asbestos-Associated Cancers", *Cancer Investigation*, vol. 14, no. 5, pp. 466-480, 1996.

[47] S. Zhou, A. Treloar and M. Lupien, "Emergence of the Noncoding Cancer Genome: A Target of Genetic and Epigenetic Alterations", *Cancer Discovery*, vol. 6, no. 11, pp. 1215-1229, 2016.

[48]G. Goodman, "Prevention of lung cancer", *Critical Reviews in Oncology/Hematology*, vol. 33, no. 3, pp. 187-197, 2000.

[50] J. Cummings and S. Bingham, "Fortnightly review: Diet and the prevention of cancer", *BMJ*, vol. 317, no. 7173, pp. 1636-1640, 1998.

[51] R. Newton and D. Galvão, "Exercise in Prevention and Management of Cancer", *Current Treatment Options in Oncology*, vol. 9, no. 2-3, pp. 135-146, 2008.

[52] A. Argiris, M. Karamouzis, D. Raben and R. Ferris, "Head and neck cancer", *The Lancet*, vol. 371, no. 9625, pp. 1695-1709, 2008.

[53]R. Arriagada, F. Eschwege, Y. Cachin and J. Richard, "The value of combining radiotherapy with surgery in the treatment of hypopharyngeal and laryngeal cancers", *Cancer*, vol. 51, no. 10, pp. 1819-1825, 1983.

[54] G. Tirelli et al., "Tailored resections in oral and oropharyngeal cancer using narrow band imaging", *American Journal of Otolaryngology*, vol. 39, no. 2, pp. 197-203, 2018.

[55] P. ROBIN, D. POWELL and J. STANSBIE, "Carcinoma of the nasal cavity and paranasal sinuses: incidence and presentation of different histological types", *Clinical Otolaryngology*, vol. 4, no. 6, pp. 431-456, 1979.

[56] M. Gillespie, W. Albergotti and D. Eisele, "Recurrent Salivary Gland Cancer", *Current Treatment Options in Oncology*, vol. 13, no. 1, pp. 58-70, 2012.

[57] J. Bruce, K. Yip, S. Bratman, E. Ito and F. Liu, "Nasopharyngeal Cancer: Molecular Landscape", *Journal of Clinical Oncology*, vol. 33, no. 29, pp. 3346-3355, 2015.

[58]S. Duffy, J. Terrell, M. Valenstein, D. Ronis, L. Copeland and M. Connors, "Effect of smoking, alcohol, and depression on the quality of life of head and neck cancer patients", *General Hospital Psychiatry*, vol. 24, no. 3, pp. 140-147, 2002.

[59] R. Khlifi and A. Hamza-Chaffai, "Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review", *Toxicology and Applied Pharmacology*, vol. 248, no. 2, pp. 71-88, 2010.

[60] L. Marrett, "Prevention of Skin Cancer", *Cancer Causes & Control*, vol. 15, no. 3, pp. 329-330, 2004.

[61] H. Nakagawa et al., "The targeting of the cyclin D1 oncogene by an Epstein-Barr virus promoter in transgenic mice causes dysplasia in the tongue, esophagus and forestomach", *Oncogene*, vol. 14, no. 10, pp. 1185-1190, 1997.

[62] J. Werbrouck et al., "Single-nucleotide polymorphisms in DNA double-strand break repair genes: Association with head and neck cancer and interaction with tobacco use and alcohol consumption", *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, vol. 656, no. 1-2, pp. 74-81, 2008.

[63] J. van der Pols, G. Williams, N. Pandeya, V. Logan and A. Green, "Prolonged Prevention of Squamous Cell Carcinoma of the Skin by Regular Sunscreen Use", *Cancer Epidemiology Biomarkers & Prevention*, vol. 15, no. 12, pp. 2546-2548, 2006.

[64] N. Beech, S. Robinson, S. Porceddu and M. Batstone, "Dental management of patients irradiated for head and neck cancer", *Australian Dental Journal*, vol. 59, no. 1, pp. 20-28, 2014.

[65] S. Waggoner, "Cervical cancer", *The Lancet*, vol. 361, no. 9376, pp. 2217-2225, 2003.

[66] E. Franco, T. Rohan and L. Villa, "Epidemiologic Evidence and Human Papillomavirus Infection as a Necessary Cause of Cervical Cancer", *JNCI Journal of the National Cancer Institute*, vol. 91, no. 6, pp. 506-511, 1999.

[67] A. PHILLIPS and G. SMITH, "Cigarette Smoking as a Potential Cause of Cervical Cancer: Has Confounding been Controlled?", *International Journal of Epidemiology*, vol. 23, no. 1, pp. 42-49, 1994.

[68] E. Burd, "Human Papillomavirus and Cervical Cancer", *Clinical Microbiology Reviews*, vol. 16, no. 1, pp. 1-17, 2003.

[69] R. Bernhardt, "Cytochromes P450 as versatile biocatalysts", 2020.

[70] J. Butel, "Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease", *Carcinogenesis*, vol. 21, no. 3, pp. 405-426, 2000.

[71] K. Mitrunen and A. Hirvonen, "Molecular epidemiology of sporadic breast cancer", 2020.

[72] V. Ovsepyan, E. Vinogradova and E. Sherstneva, "Cytochrome P4501A1, glutathione S-transferase M1 and T1 gene polymorphisms in chronic myeloid leukemia", 2020.

[73] Syvänen, A. C. (2001). Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nature Reviews Genetics*, *2*(12), 930-942.

[74] Brumfield, R. T., Beerli, P., Nickerson, D. A., & Edwards, S. V. (2003). The utility of single nucleotide polymorphisms in inferences of population history. *Trends in Ecology & Evolution*, *18*(5), 249-256.

[75] M. Esteller, "Non-coding RNAs in human disease", 2020.

[76] J. Butel, "Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease", *Carcinogenesis*, vol. 21, no. 3, pp. 405-426, 2000.

[77] J. Mullikin et al., "An SNP map of human chromosome 22", 2020.

[78]S. Fattahi et al., "Cytochrome P450 Genes (CYP2E1 and CYP1A1) Variants and Susceptibility to Chronic Hepatitis B Virus Infection", *Indian Journal of Clinical Biochemistry*, vol. 33, no. 4, pp. 467-472, 2017.

[79]K. Lee et al., "Paternal smoking, genetic polymorphisms in CYP1A1 and childhood leukemia risk", *Leukemia Research*, vol. 33, no. 2, pp. 250-258, 2009.

[80]C. Villanueva et al., "Coffee consumption, genetic susceptibility and bladder cancer risk", *Cancer Causes & Control*, vol. 20, no. 1, pp. 121-127, 2008.

[81]R. Li et al., "Common genetic variations of the cytochrome P450 1A1 gene and risk of hepatocellular carcinoma in a Chinese population", *European Journal of Cancer*, vol. 45, no. 7, pp. 1239-1247, 2009.

[82]T. Lam et al., "Dietary quercetin, quercetin-gene interaction, metabolic gene expression in lung tissue and lung cancer risk", *Carcinogenesis*, vol. 31, no. 4, pp. 634-642, 2009.

[83] "Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang, J.G. and Osborn, T.C. (1994) Comparison of RFLP and RAPD Markers to Estimating Genetic Relationship within and among Cruciferous Species. Theoretical and Applied Genetics, 88, 973-980.

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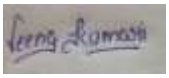

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