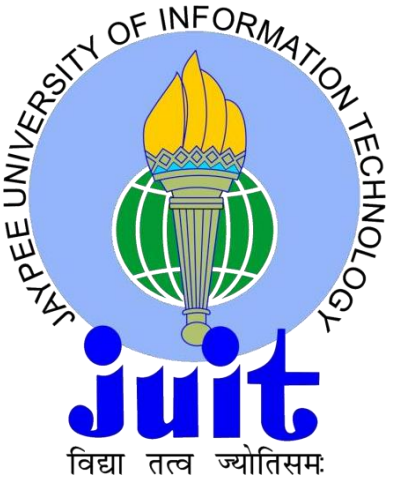
FDFT1 Variant rs2645429 and Cancer Susceptibility in Himachal Population

Project report submitted in partial fulfillment of the requirement for the Degree of
Bachelor of Technology

in Biotechnology Submitted by

Siddhant Singh (151805)



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May- 2019

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CERTIFICATE

This is to certify that the work titled "**FDFT1 Variant rs2645429 and Cancer Susceptibility in Himachal Population**", submitted by **Siddhant Singh** in partial fulfillment 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

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Acknowledgement

I sincerely am thankful and grateful to my guide Dr. Harish Changotra for his extraordinary and brilliant guidance, constant and valuable feedback along with his continuous encouragement throughout the duration of my major final year project. I am very thankful to him for providing me this wonderful opportunity to work in his esteemed lab.

I am highly thankful to Ms Sargeet Kour for her constant support during my project work and for her continuous guidance, mentorship and inspiration.

I am also very thankful for Dr. Sudhir Syal for his valuable assistance and guidance and providing liberty to do good and quality experimental work.

SUMMARY

Cancer is one of the leading causes of death all around the globe and the latest data states that around 18 million people have cancer and out of them around 2 million dies every year. Although a lot of studies have been performed and a lot are still going on in order to find out the root cause of a cell to become cancerous and cause this deadly infection in the body and certainly lead to death and to find better options for the treatment of the cancer.

Although multiple factors are the coin reasons for the development of cancer based upon the environmental factors, genetic factors and lifestyle factors. But finding out the hereditary pattern can be an important milestone in finding the inheritance pattern of cancer. Here in this study we have been using the reference from the pattern of single nucleotide polymorphism of rs2645429 in order to find out its relationship with the gene FDFT1 and its susceptibility to the common cancer among the Himachal Pradesh population.

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INTRODUCTION

Cancer is a disease causing condition in which there is an involvement of abnormal and uncontrolled cell growth which might spread in other different parts of the body from one potential affected region to other. Humans and animals have had cancer since a very long period of time although it was not called cancer at that time. The term cancer was termed by the famous Greek physician "Hippocrates" who used the term carcinos and carcinoma to describe the non-ulcer forming and ulcer forming tumors. In a cancer affected body the detection can be done easily due to presence of tumor, except in case of leukemia. As there is presence of natural growth maturation and death of every cell in the body (every molecule has its decline phase in the universe) cancerous cells on the other hand lacks this capability and continues to grow and multiply rapidly [1, 2] . This uncontrolled growth and lacking of the decline phase in the usual life cycle leads to the multiplication of a million cells from just few cells in a very short span of time. Certainly with rapid growth there is a huge competition of for the nutrients and oxygen between the normal and cancerous cells leading to very fast exhaustion of the energy in the body leading to many other conditions such as weakening of the body's immune responses and other irregularity in the proper function of the body [1, 2].

If we consider about the total number of all cancer cases that have occurred per year it goes up to a dangerous number of around 18 million people suffering from some kind of cancer and in India itself we have about 1.5 million cases of cancer contributing to almost 6.4% of all cancer cases around the globe. With a shocking mortality rate of around 9.5 million deaths as of 2018 and in India with approximately 780,000 people dying from cancer as of December 2018 [3].

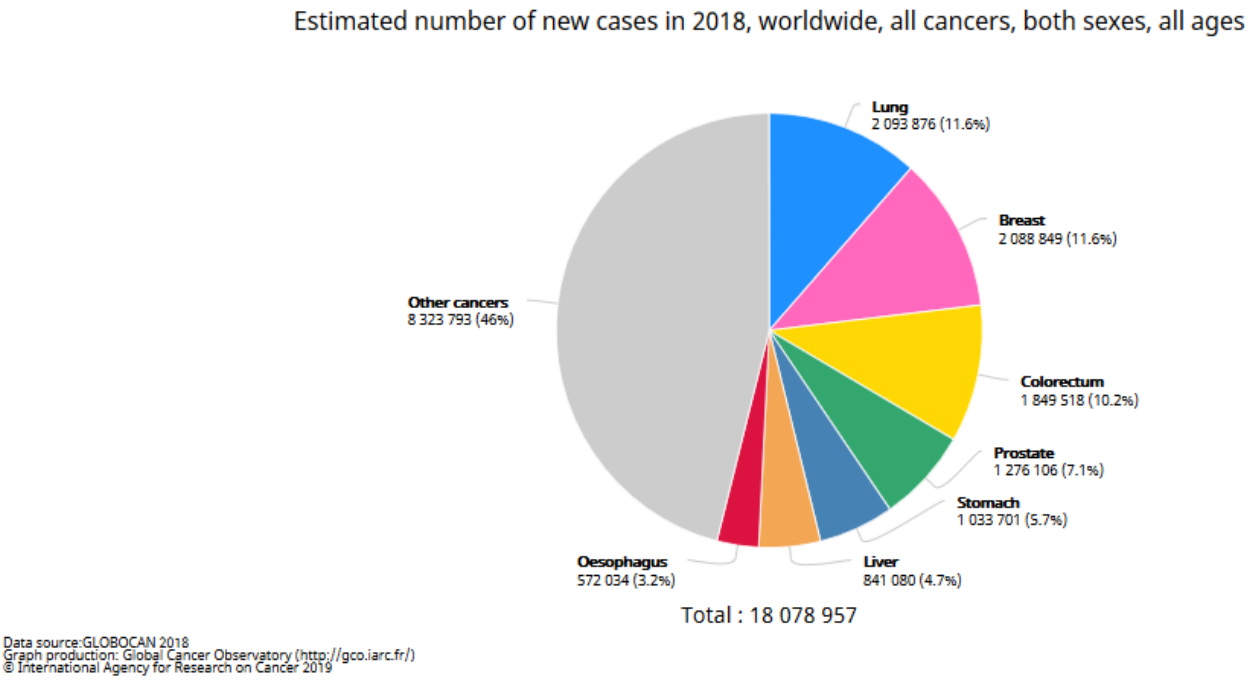


Figure 1: Global Statistics of Cancer according to latest WHO and GLOBOCAN database; Ref-GLOBOCAN 2018 Graph production: Global Cancer Observatory (http://gco.iarc.fr/), International Agency for Research on Cancer; 2019

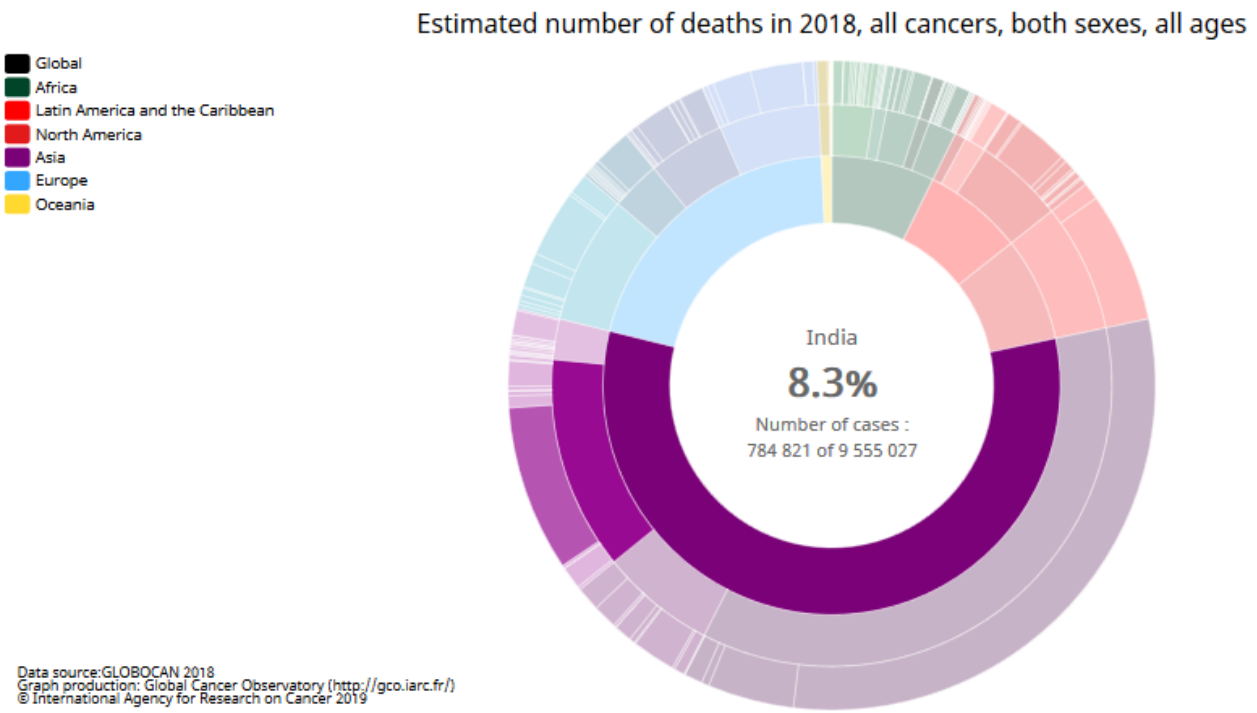


Figure 2: Stastistics about death rate caused by Cancer globally; GLOBOCAN 2018 Graph production: Ref- Global Cancer Observatory (http://gco.iarc.fr/); International Agency for Research on Cancer; 2019.

From over more than 120 types of cancer, most common types of cancers that are among most of the cases and are the reason of deaths are-

Some common types of cancer -	
Bladder Cancer	Lung Cancer
Breast Cancer	Pancreatic Cancer
Colon and Rectal Cancer	Prostate Cancer
Kidney Cancer	Stomach
Leukemia	Liver

And more other types of cancer that are classified on the basis of their susceptibility to the gender and age present leading to the chronic conditions [3,4].

Genes that are involved in the process of carcinogenesis are mainly classified into two types – The oncogenes and the tumor suppressor genes. Mutations and further epigenetic changes in these oncogenes and tumor suppressor genes can be considered as the major reasons for initiating the carcinogenesis and causing cancer [5,6]. Generally there can be more than one type of mutations including missense, frameshift or nonsense mutation. Although it is not necessary that only the mutations affecting amino acids are responsible for carcinogenesis initiation , sometimes mutations effecting the promoters and splicing sites also lead to the condition [7].Among various methods responsible for mutations some of the most potent ones can be deletion of small or large DNA segments, inversions ,translocations , or looping leading to truncated sequences. There can be various numbers of reasons for these type of transformations such as chemical or radioactive carcinogens but in case of cancer there are no defined reasons [8].

If we talk about the genetics of the cancer and other risk related factors that might lead to cancer due to hereditary transfer from the previous generations, there are usually around one in every ten cases of cancer that are associated with hereditary predisposal but on conclusion cancer is not inherited. Which indeed also has the other side of this fact is that the families or certain group do not poses the predisposition in hereditary traits that might lead to cancer. Cancer on account is a collective result of various factors such as environmental combined with hereditary effects. Since the genes that inherited to the family members can only lead to develop the cancer or maybe the risk factors that will lead to development of cancer. But, all those who will have same gene might

not have the cancer or those who don't might develop the cancer in their body. Hereditary cancer or inhibition of such factors that will lead to cancerous condition in the body can be identified on the early onset of the cancer at an early age development of same type of cancer as the other person in the family [9]. Usually the genetic disposition of the autosomal dominant type imposes a higher risk factor for developing such condition and other types of cancer as well. So far now more than 30 types of such hereditary cancer have been found being mutant or present in heterozygous condition [10]. In order to find out the key aspects of the hereditary factors for cancer we can look at the protein coding region exclusively from the genome to identify the variation in expression.

The non-protein coding region of the genome serves as a template for the transcription of various non-coding RNAs [11]. There are two types of RNA non coding regions: small non-coding RNA and the long non coding RNA. LncRNAs are found in antisense or sense orientation to protein coding regions of the genome and can function in cis (major) and trans as well [12]. It includes a diversified ways of mechanisms by which the LncRNAs effect in the process of carcinogenesis but the studies show that their major role is to guide the site of specificity to the chromatin-modifying complexes to cause changes at epigenetic levels [13].

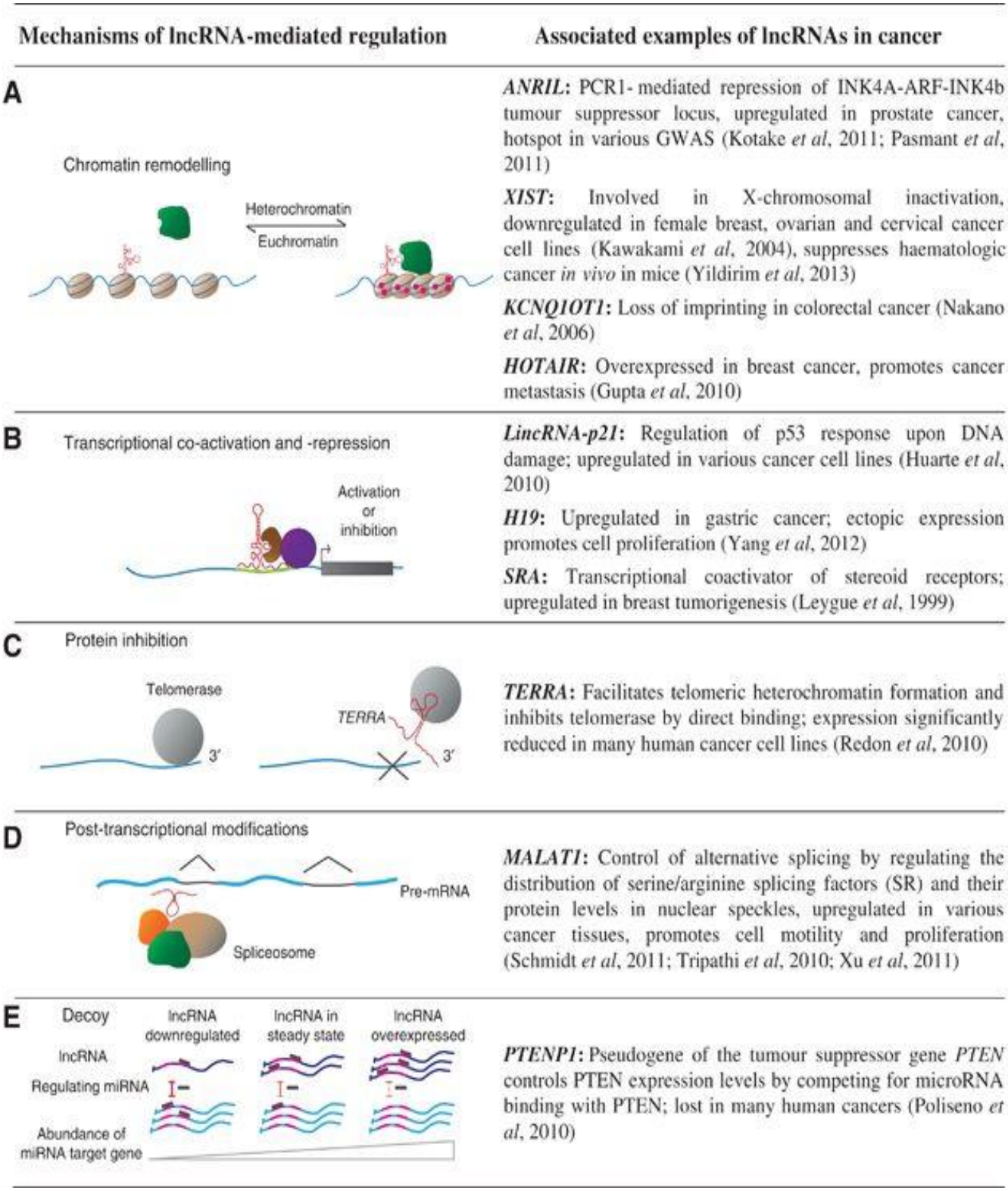


Figure 3 : Cancer association and causing risks by LncRNA; Cheetham, S. W., F. Gruhl, J. S. Mattick, and M. E. Dinger. "Long noncoding RNAs and the genetics of cancer." British journal of cancer 108, no. 12 (2013): 2419.

The misexpression of LncRNAs have lead to the development of cancers and enter in to carcinogenesis and diverse it more. Some well characterized lncRNA are: ANRIL, XIST, HOTAIR and KCNQ1OT [14]. There are majorly 5 types of approaches by which the lncRNA can affect in progressiveness of the cancer; by chromatin remodeling, by transcriptional co-activation and repression, protein inhibition, post transcriptional modifications and competing for microRNA by binding with the PTEN gene [15].

Here in this study we will be going to identify the possible genetic mutations that are present in the population of Himachal Pradesh for the gene Farnesyldisphosphate Farnesyltransferase-1 (FDFT1) gene and its susceptibility in association with common type cancers like Lung cancer , Head and Neck (Oesophagal) Cancer, and Cervical Cancer.

[2.]REVIEW OF LITERATURE

[2.1] Cancer-

The most commonly used definition of cancer is, it is a disease that lead to the abnormal growth along with rapid and uncontrolled division of the cells that are prone to get spread in other parts of the body [16]. Since every cell in the human body has to follow the general life cycle of becoming adult, undergoing division, and after ageing they have to die. But in case of cancer cell this simple and necessary rule does not follows due to which abnormalities in the body occurs since the old and damaged cells do not undergo their senescent phase and instead of no requirement the new cells keep on developing. This leads to formation of masses of tissues known as tumors. Many cancer cells form tumors except in the case of leukemia [17]. Tumors are mainly of two types: Benign and Malignant tumors.

Benign tumors are non cancerous tumors that won't harm the nearby cell and the usual cell growth and division. They are not that lethal except in some cases where they can be present at critical spots of the body such as nerves, blood vessels, brain, etc. Since there is possibility that the benign tumors can eventually start the process of metastasizing and might develop cancer they are supposed to be removed with the help of surgeries. On the other hand Malignant type tumors are the ones that are cancer causing and might spread into different parts of the body via veins, lymph nodes or blood vessels and undergo metastasis [18,19]. Metastasis or the spreading of cancer mainly is processed through blood vessels or lymph nodes into different parts of the body. Since the environment that the metastatic cells required are not always favorable so they tend to die many times but as long as the conditions are favorable they tend to process step by step and form tumors in different parts of the body. Although it is not necessary that the metastatic cells creates tumor every time, there are some cases in which the cells tend to remain inactive in some parts of the body for several years. The most common region in the body where cancer can spread are bone, liver and lung [20].

Types of cancer

If we talk about the types of cancer there are various classifications that have been done on the basis of the region where it occurs or the organ it affects, but the doctors have classified them into 4 major groups- **Carcinoma, Sarcoma, Leukemias, Lymphomas.**

1. **Carcinoma**- It is the type of cancer that usually arises from the epithelial cell lines, i.e., from the organs that have tissue lining organs such as liver or kidneys [21]. The carcinomas usually grow and divide in an uncontrolled manner. Carcinoma are further classified into other sub categories-

- Basal Cell Carcinoma
- Squamous Cell Carcinoma
- Renal Cell Carcinoma
- Ductal Carcinoma in Situ
- Invasive ductal Carcinoma
- Adenocarcinoma

2. **Sarcoma**- It is a type of malignant cancer that occurs in the connective tissues (Bones, cartilage, muscles, etc.) [22,23]. They can occur in many parts of the body such as bones , tendons , muscles , cartilage , nerves , etc. and may spread to other parts also. With more than 50 types of sarcoma, this type of cancer is majorly grouped into two classifications, **Soft tissue Sarcoma** and **Bone sarcoma** (osteosarcoma) [24].
3. **Leukemia**- The type of cancer that occurs in the bone marrow and then spreads to the other parts of the body by uncontrolled division and proliferation of abnormal blood cells [25]. Such blood cells usually face the abnormality of not fully grown and are therefore called blasts [26]. And is categorized into two different types based on the severeness and the time period of the disease lasting- **Acute Leukemia** and **Chronic Leukemia.**
4. **Lymphomas**- The type of cancer that occurs in the infection-fighting cells of the immune system known as lymphocytes (type of WBC), in which the lymphocytes changes and starts dividing in uncontrolled manner. There are two major types of lymphomas, Non-Hodgkin (most of lymphomas are of this type) and Hodgkin [27].

Causes of Cancer-

There are many causes and reasons that lead to the development of cancer in human body based upon the environmental factor , individual immunity , type of the infection , Genetic factors , food and daily activities , drug uses , and other risk factors. Cancer is caused by molecules known as carcinogens and they lead to carcinogenesis and finally development of the cancerous cells.

Although there is never only one factor that will lead to the development of cancer inside the body of a person, there will the multiple factors that shall lead to such a devastating collective result and developing cancer. Major cancer development happens due to the occurrence of mutations in the body by the influence of genetic as well as environmental factors [28].

Further the risk associating factors of cancer can be divided into the following categories-

- **Biological** factors or the Body influenced factors that can include the immunity, the age, sex, genetic influences or mutations, etc.
- **Environmental** related factors can contribute being the major risk association that leads to cancer, such as pollution, exposure to fine particulate matter environment, exposure to radiation such as radon or UV, exposure to carcinogenic smoke, exposure to unhygienic living conditions, etc.
- Occupational environment or the **work environment** in which a person works also influences cancer, such as the exposure to chemical carcinogens in industries, carcinogens in form of aerosols that might lead to other conditions except than cancer, exposure to radiations, etc. some work related risk factors can include, asbestos fibers, tar & pitch, poly nuclear hydrocarbons, plastic chemicals and other metal compounds can also associate to risks of having cancer.
- Another major influencer of cancer can be the way of **lifestyle** in which one lives and his surroundings bases upon the level of cleanliness and hygine, drinking water, air quality, particulate matter in the air nearby ,etc. Some lifestyle related factors can be tobacco, alcohol, UV from sunlight, food and nutrition related factors.

Except from these major factors there can be other factors that might lead to development of cancer in the body such as the bacterias or the viral pathogens that affects the body to cause some disease and the repercussions of those lead to development of cancer tumors in the body, like HPV (Human papilloma virus), HCV&HBV (Hepatitis virus), EBV (Epstein-Barr Virus).

Other contributing factors that can be causative agents for cancer can be Radiation. Exposure to ionizing (X-ray, soil radon) and non-ionizing (Sun's UV radiation) type radiation might lead to cancer. Cancer is a condition in which if developed the person remains in pain during the treatment (if possible) but it can even be developed by over use of certain drugs such as ingestion of antineoplastic agents, or any medicines that might cause deficiency in the immune system [29].

Since we have performed the study on 3 different types of cancer that are found in the population of Himachal Pradesh-

1. **Lung Cancer**
2. **Head and Neck Cancer**
3. **Cervical Cancer**

The above are explained in the later part of this study respectively.

[2.2]Lung Cancer-

It is the malignant type of tumor that happens to occur in the lungs leading to uncontrolled growth and division of cellular masses in the body and might spread to different parts of the body , which is why it is also known as lung carcinoma [30,31]. There are two main types of lung cancer-

- Small Cell lung Cancer
- Non Small Cell Lung Cancer

Small Cell Lung Cancer (SCLC) - It is the type of malignant lung cancer found in the tissues of the lungs and it causes around 10-15% of all the lung cancer that occurs. Further SCLC can be divided into two sub categories- Small cell carcinoma (Oat cell Carcinoma) & combined small cell carcinoma [32].

Non-Small Cell Lung Cancer (NSCLC)- It is a collective term used to indicate all other types of the cancer that are different from small cell lung cancers. NSCLC are 80-85% of cases of lung cancers and is more lethal one. It can be further classified on the basis of the carcinoma that it can cause– squamous cell carcinoma, adenocarcinoma and large cell carcinoma.

Causes and Risk Factors-

Although there are a multiple factors that can be associated with the susceptibility of having lung cancer including environmental, genetics , lifestyle , etc., and the risk factors that can be associated with chances of development of along carcinoma in the body. Some common risk factors can include-

1. **Smoking**- Smoking is one of the leading causes of lung cancer, whether it is active or passive it certainly will lead to the production of carcinogens in your body and might lead to development of malignant type lung cancer. Being the main reason of contributing to the lung cancer, the repercussions of getting cancer after smoking depends upon the type of tobacco that one smokes and for how long time the person was smoking that. Tobacco has more than 73 types of known carcinogens that can lead to such condition. It can be avoided by stopping to smoke, as soon as it is done, the higher will be the chances to avoid lung cancer [33,34].

2. **Exposure of radon Gas**- Radon is a gas that's being produced by the decay of uranium with time and is found in the inner part of the earth. Since it's a radioactive bi-product so that might ionize and mix up in the soil and water leading to further mutations at genetic level by damaging the genetic material leading to development of cancers [35].
3. **Exposure to asbestos and other carcinogens**- Exposure to harmful carcinogens such at workplaces or from industrial neighbors can also lead to development of lung cancer. Carcinogens such as asbestos, nickel, chromium, arsenic, etc., can increase the chances of lung cancer by 45-folds [36].
4. **Family History** – Hereditary causes of lung cancer and other types of cancer are under good research and so far now there have been many proved studies that show that familial history of having lung cancer might lead to the twice the chances of increased risk of having lung cancer since multiple genes are included in it [37].
5. **Genetics**- Mainly the polymorphism or alteration of some nucleotide in the DNA sequence on particular chromosomes such as 5, 6 and 15 are the major ones for the development of the lung cancer. SNPs encoding for the genes such as CHRNA5, CHRNA3 and CHRNB4 along with RGS17 are some which have increased association of lung cancer risk [38].

Epidemiology-

Statistics about lung cancer says that out of all the cases of cancers, 11.6% of the case are of lung cancer and out of those ~67,000 cases are from India itself contributing to 3.3% of all lung cancer. And out of total cancer deaths ~1.7 million deaths are caused by lung cancer and in India 3.6% deaths are caused by lung cancer out of all the cancer cases [39].

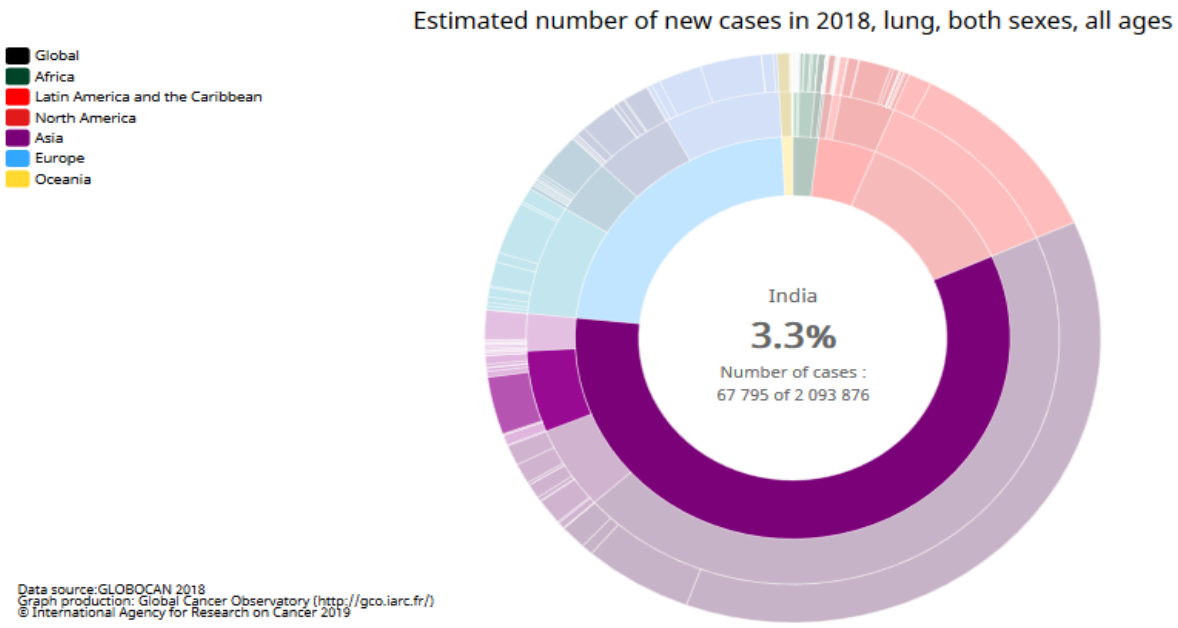


Figure 4: Lung cancer statistics around the Globe; GLOBOCAN 2018 Graph production: Global Cancer Observatory (http://gco.iarc.fr/); International Agency for Research on Cancer; 2019

Staging-

Staging of the cancer indicates the severity of the cancer, that how lethal the infection is in the body and by how far the cancer has metastasize or spread in the other parts of the body. Since the staging definitions may vary, but in clinical terms staging is differentiated into following categories-

Occult or Hidden- The cancer cells are present in very low amount in the mucus but they are not detected in the scans and imaging.

Stage 0- Doctors tend to find the cancerous cells in the upper layer only.

Stage 1- The size of the developed tumor in the lung is lesser than 5cm and is not spread into other parts of the body.

Stage 2- Tumor is usually between size 5-7cm but has spread into the lymph nodes in areas of lung and other tissues nearby the lung.

Stage 3- the cancer has reached to other parts of the lungs and into the lymph nodes of lung.

Stage 4- Cancer has already spread to other parts of the body [40,41].

Treatment-

Possible treatment of the cancer includes many practices but major ones can be-

1. Surgery
2. Chemotherapy
3. Radiation Therapy
4. Targeted Therapy

Preventions from Risk factors to get Lung Cancer-

1. **Stop Smoking**- Tobacco smoking is still the leading cause of lung cancer and is the main reason that this disease goes into metastasis so easily and spread to other parts of the body. So if a person hasn't smoke ever he should not smoke at all and the ones who do it should leave it as soon as possible.
2. **Avoid Passive Smoking**- Passive smoking or second hand smoking is also a leading reason of the lung cancer, since many studies have proven that passive smoking is more lethal than the active ones, Since it has long term effects and is more dangerous for the children in growing ages since in long term it might lead to behavioral and psychological changes also.
3. **Eat healthy Diet**- One of the best ways to avoid any type of disease can be taking a good and nutritious diet that can help maintain the immunity of the body. Everyday intake of good vitamins and antioxidants can help in eradicating the harmful carcinogens from the body keeping you safe from development of any kind of infection.
4. **Avoid Carcinogens at work**- Take proper care and protective measures while working in an environment where carcinogens are there and follow all the safety measures and instructions like wearing masks, gloves, washing hands before and after eating, etc.
5. **Exercise**- One of the most effective and efficient way to stay healthy can be by doing physical workout every day. One must perform enough amount of exercise in any terms such as playing, yoga, cross-fit, jogging etc. These helps in detoxifying the body of the carcinogens and produce good immunity in the body and avoiding such harmful infections [42-48].

[2.3]Head And Neck Cancer-

These are a collective group of different malignant type cancers that occurs in the squamous cells found in mucus and moist regions of mouth, throat, larynx, or salivary glands [49]. Mainly squamous cell carcinoma is the type of cancer that develops in the regions of head and neck such as – Oral cavity, Pharynx, Larynx, Paranasal Sinuses and nasal cavity, Salivary glands. Further the head and neck cancer are divided into 5 major groups based upon the regions where the carcinoma occurs-

- Laryngeal or Hypopharyngeal Cancer
- Nasopharyngeal Cancer
- Nasal cavity and paranasal sinus Cancer
- Oral and Oropharyngeal Cancer
- Salivary Gland Cancer

1. **Laryngeal or Hypopharyngeal Cancer**- It is the type of cancer that occurs in the larynx or the Voice box. Laryngeal cancer is a type of malignant cancer that occurs in the squamous cells of glottis of the larynx. Since it is a type of malignant cancer so it spreads though the lymph nodes from one part to other parts of the body. When the carcinoma occurs at the junction point where larynx and oesophagus meet, i.e, the pharynx it is called as the hypopharyngeal cancer [50,51].
2. **Nasal Cavity and Paranasal Sinus Cancer**- It is the type of malignant cancer that occurs in the paranasal region and in the sinus region where the mucus is produced. Squamous cell carcinoma is the most common type cancer that occurs in the nasal cavity, but there are also other forms of cancer that tends to affect these regions like- Melanoma, Sarcoma, Inverting Papilloma, Midlinegranulomas [52].

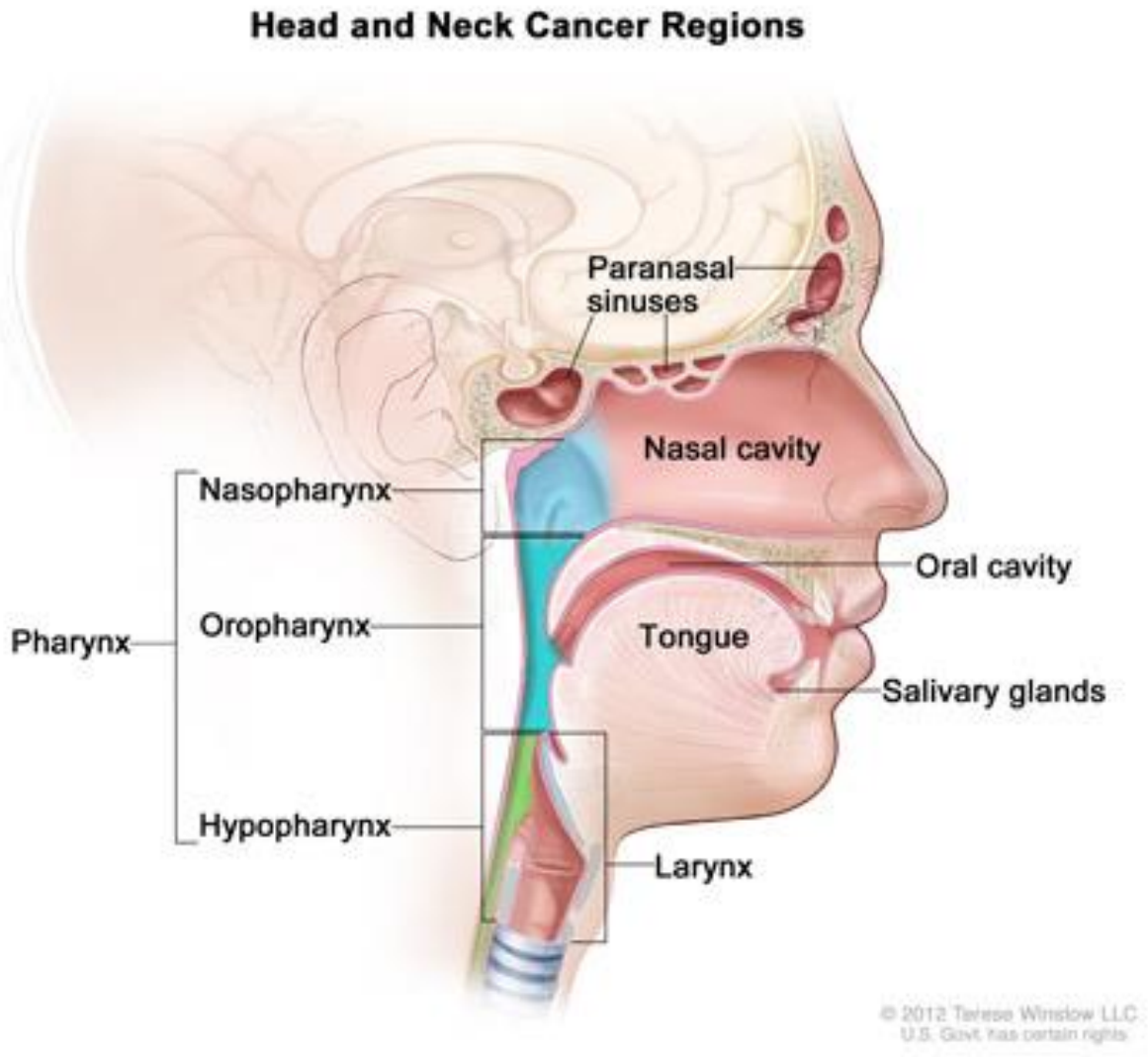


Figure 5: Region where Head and Neck Cancer occurs usually; Ref- *"Head and Neck Cancers". NCI. March 29, 2017. Retrieved 17 September 2017.*

3. **Nasopharyngeal Cancer**- It is one of the major type of HNC and it occurs in the nasopharynx in the upper region of the throat and behind the nose. Also known as Nasopharyngeal Carcinoma (NPC).
4. **Oral and Oropharyngeal Cancer**- It is the type of cancer that occurs in the mouth or the oral and the oropharynx the region just behind the mouth. Usually the carcinoma type that affects this part is the squamous cell carcinoma, but other type of benign tumors can also affect this region of the Head and neck portion. These two are the most common type of cancer that occurs in the head and neck region [54].
5. **Salivary Gland Cancer** – It the type of cancer in which malignant cancerous cells occur in the saliva producing salivary gland. Out of 3 major pair of salivary gland the parotid gland which is the largest one is most likely one to develop the tumor of malignant cancer [55,56].

Causes and Risk Factors-

Although a number of studies have proven that HNC is caused by a combination of multiple reasons and risk factors but main reason is the alcohol and tobacco abuse. Overuse of such carcinogenic product leads to development of benign or malignant type tumors. Other such factors that can cause HNC include the genetic, environmental or lifestyle habits. People who are infected with cancer causing HPV (Human Papilloma virus) have increased risks of getting head and neck cancer [57-60].

Other related risk factors of Head and neck cancer are-

- **Paan (Betel quid)** -Studies have shown that the people from the south east Asia who intake betel quid in excess amount have been found more prone to get such cancer and most of them can be malignant [61,62].
- **Preserved or Salted foods**- Eating of junk foods that are preserved and have been salted by chemicals that might become carcinogenic can be very dangerous for the children of earlier ages since it can lead to development of nasopharyngeal cancer [63,64].

- **Oral Health**- A good and healthy mouth have lesser chances of developing Oral cancer , but in case of carelessness, there is a higher risk that malignant cells might form in the salivary gland or in the oral cavity [65,66].
- **Work Exposure**– Many studies shows that people who are exposed to the carcinogenic work environment have greater risk of having nasopharyngeal cancer. Although exposure to asbestos , wood , dust and other aerosol can lead to greater risk of larynx cancer and the ones who are in exposure to formaldehyde or nickel; dust are more prone to get paranasal sinus cancers [63-64,67-69].
- **Radiation**- exposure in to radiations such as inn labs or sterilizing work stations can lead to formation of carcinomas in the salivary gland [65].
- **EBV infection** – Person who are suffering from an infection caused by EBV virus have greater risks of getting nasopharyngeal or salivary gland cancer [70].
- **Ancestry**- Hereditary factors can also be possible for the person to get HNC. A person with familial history of HNC can have higher risk of having the certain type of HNC [63,64].

Epidemiology-

According to the latest GLOBOCAN data and analysis the epidemiology of different HNC is based on the types of cancer and the region where they are affected.

Out of ~80,000 cases of hypopharynx cancer globally, 32.8% of those cases are from India making it the maximum in the world. On the other hand talking about Larynx cancer, India again stands at the first place with having ~29,000 cases out of total ~177,000 cases globally. When it comes to oral cancer India is having the maximum number of cases, out of nearly 350,000 cases globally India has 119,000 cases from all. In case of nasopharynx cancer India has only 4% cases from the global level of collective cases of ~130,000 cases. For oropharynx cancer India stands number 1 in the world with 17000 cases out of all 92000 cases of oropharynx cancer globally. If we talk about salivary gland cancer India stands 2nd there with having ~7600 cases out of total 52000 cases globally [71].

Diagnosis of Different Head and Neck Cancer –

Diagnosis of HNC is very important since the mortality rate in the later stages tends to reduce by many folds. So for early detection of such cancer there are various disagnostic techniques such as-

1. Physical examination and Urine Tests
2. Endoscopy
3. Biopsy
4. Molecular testing of tumor
5. X-ray/ Barium swallow
6. Panoramic radiograph
7. Ultrasound
8. CT-Scan
9. Magnetic Resonance Imaging
10. Bone Scan
11. PET CT Scan

Treatment Options-

Since its a very severe type of chronic infection that can lead to death if not taken care of very seriously and effectively. Following treatment options are most common ones-

- Surgery
- Radiation Therapy
- Chemotherapy
- Photodynamic Therapy
- Targeted Therapy

Since the research and studies are still going on in order to find the true and most effective treatment of HNC [72,73].

Prevention-

Prevention from the risks of having any type of HNC can be the most effective way of avoiding your chances to have such carcinomas.

1. **Stopping Substance Abuse**- Tobacco and its byproducts have been the leading causes of the HNC , avoiding their usage and making people aware about its ill effects can help avoiding such conditions and fight against this deadly disease.
2. **NO Alcohol**- Another major reason for most of the HNC cases is alcohol. Alcohol creates toxicity in body leading to formation of highly lethal carcinogens that might develop cancer in larynx or oral cavity. Stopping the consumption of alcohol and its beverages can help in reducing the risks of getting such deadly cancers.
3. **Regular Dental checkups**- To avoid any chances of getting such chronic disease, regular appointments at dentists can help in avoiding formation of any unwanted plaques or later formation of tumors in the oesopharynx region.
4. **Good Oral Hygiene**- can be another major beneficial step that one can take to avoid having such carcinoma. Regular brushing of teeth and using sterilizing mouth washes everyday can be a good and effective step [50].

[2.4]Cervical Cancer-

The cancer that occurs from the cervix and the cells grow abnormally and their division becomes uncontrolled due to which it also spreads to other parts of the body such as, bladder, lungs, liver, vagina and rectum. Cervical cancer is a slow progressive type of cancer which gives the patients enough time to take actions an possible treatments for it. Due to its property of slow progression there are enough opportunities for the early diagnosis and avoid the situation of metastatis in the carcinoma. Cervical cancer is a collective result of precancerous stages over a long period of time. Cervical cancer is categorized by the type of cells that tend to form at the cervical regions- Squamous cells carcinoma (90%) and Adenocarcinoma (10%) [74-78].

Causes-

Since development of cancerous cells leads to formant of cell masses at a place and then tend to go under metastasis and the doctors still don't know why this phenomenon occurs. Cervical cancer has many reasons since its prognosis is slow so it will develop slowly but with multiple affects it can fasten up. Following are the major causes of Cervical Cancer-

1. **HPV (Human Papilloma Virus)-** Even though many studies show that for the development of cervical cancer , HPV infection is must. HPV has more than 100 types of viral strain out of which 15 are the ones that can lead to the formation of cervical cancer in the body. Out of all those type 16 and type 18 are the reasons for 75% of cancer cases caused by HPV. HPV leads to formation of skin warts, genital warts and other such skin related conditions that might later lead to the formation of cancer [76,79-81].
2. **Smoking**- One of the major causes of formation of cancer in the body is smoking, Carcinogenic compounds present in the cigarette tend to go and react with the cells in the cervix and might lead to formation of malignant type tumors [76].
3. **Multiple sexual partners or Early age sex**- Since while sexual intercourse there is a possibility that the same person have had intercourse with other females who can have HPV and the virus might transferred in your body leading to infection and ultimately formation of Cervical cancer. On an addition girls who tend to indulge in sexual activities at an early age (before 16) have greater chance to develop cervical cancer [76,82].

4. **Weakened Immune System**- People who have genetic mutations and have weaker immune systems are prone to get cervical cancer. Apart from those , people who are suffering from HIV or AIDS are also at greater risks and also the people who have had immunosuppressant medications for having transplants in their body are also at greater risks of having cervical cancer [76].
5. **Use of Oral contraceptive pills**- Women who take oral birth control pills have greater cancer of developing cervical cancer if the pills are taken for a longer period of time of around 4-5 years have around 3 times more chances to develop carcinomas. And those who have been taking it for more than 10 years, their chances are increased by 4 times [76,83].
6. **Multiple Pregnancies**- Women suffering from HPV and who have been pregnant for more than 5 times of full term pregnancies have greater risk of getting cervical cancer by 4 folds compare to ones have been pregnant 1-2 times [83].
7. **Other STDs** – Women who are suffering from other Sexually transmitted diseases are more prone to develop cervical cancer [82].

Diagnosis-

Diagnosis of cervical cancer help in getting rid of the tumor since early detection can be very helpful in the process of treatments due to its slow progression. Some major diagnostic techniques can include-

- Papanicolau test (Pap Test)
- Biopsy
- The loop electrosurgical excision procedure (LEEP)
- Conization
- Examination under Anaesthesia (EUA)
- CT scans
- MRI
- LLETZ
- Pelvic Ultrasound
- Cervical Intraepithelial Neoplasia (CIN)

These are some of the methods that can help in early detection and further treatment [76,82,84].

Staging of Cervical Cancer-

Staging is the process by which we can classify the severity of the cervical cancer and define whether the carcinoma has gone into the process of metastasis and to which extent it has become malignant. The standard process is the 4 staging method by which we can classify the stages of cervical cancer-

1. **Stage 0**- Precancerous cells are present that tends to undergo slow progression to develop cervical cancer.
2. **Stage 1** - Cancerous cells have been developed and reached the inner tissues of cervix and maybe in the uterus and nearby lymph nodes.
3. **Stage 2**- The cancer extends the region of cervix and uterus but not reached the pelvis or the vagina and it may affect the nearby lymph nodes also and spread to different parts.
4. **Stage 3**- Cancer has reached to the walls of pelvis or vagina and may start blocking the ureters along with affecting the lymph nodes of surroundings.
5. **Stage 4**- Cancer is now affecting the rectum or bladder and is now growing out of the pelvis. It may affect the lymph nodes and has spread to other parts of the body like bones, lungs, liver, etc [82].

Epidemiology-

Epidemiological factors are necessary to study about any disease since by help of that we can know about the possible gaps in treatments and find new options. Globally there are around 570,000 cases of cervical cancer according to latest databases. And India stages at 2nd position with around 17% cases of all cervical cancer cases globally. And the mortality rate of cervical cancer is highest in India with ~19% of all deaths of cervical cancer around the globe [71].

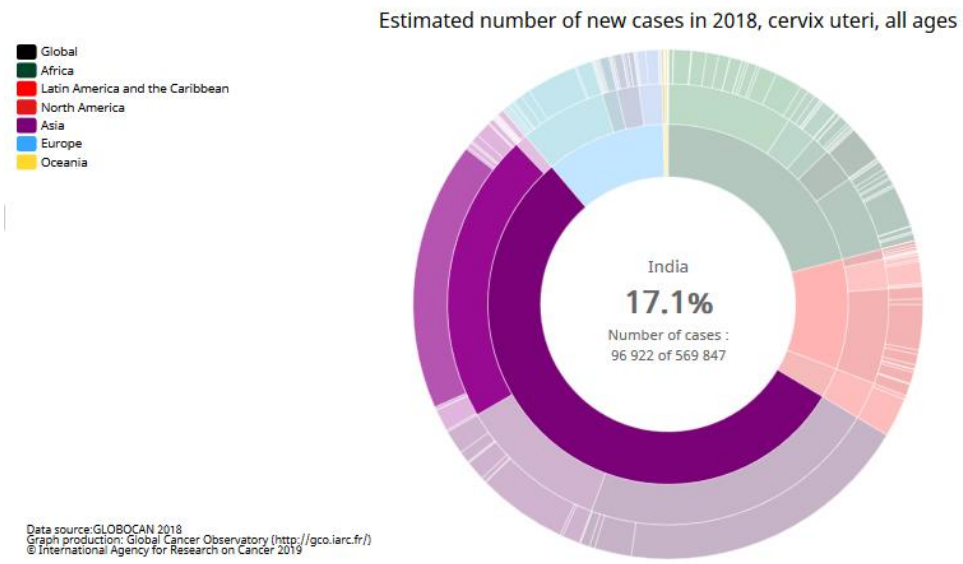


Figure 6: Global Statistics of Cervical Cancer; GLOBOCAN 2018 Graph production: Global Cancer Observatory (http://gco.iarc.fr/); International Agency for Research on Cancer; 2019.

Treatment-

With early detection it is easy for looking at the possible treatments and options. Some of the possible treatments can include-

- Hysterectomy
- LEEP or Cold Knife conization
- Cryocautery
- Laser Ablation
- Radiation Therapy
- Chemotherapy
- Biological Therapy
- Surgeries
- Implant radiation
- Brachytherapy

Prevention-

Prevention of cervical cancer can be done by taking few small steps that collectively can help one avoid the development of such carcinoma in their body. Following steps can be taken for the same-

1. **HPV Vaccine**- Since HPV infection is the major cause due to which the cervical cancer develops in the body of a woman. So vaccination for avoiding HPV can be the biggest step that one can take to avoid development of cancer [82].
2. **Stop smoking**- Smoking is one of the leading cause of cancer development , stopping to smoke can stop the formation of harmful carcinogens in the body.
3. **Safe Sex**- Practicing safe sex can help in avoiding transfer of HPV and ultimately avoiding the root cause of cervical cancer. Usage of condoms and other physical contraceptive barriers can be used for the same.
4. **Cervical Screening**- Regular screening for cervical cancer by a gynecologist can help in early detection of the cervical cancer at its precancerous stages and later go for possible treatments avoiding the possible chances of complications.
5. **Delay of First sexual Intercourse**- Early induldgement in sexual activity can lead to greater risks of getting HPV and later development of cervical cancer and its metastasis and further spread into other parts of the body.
6. **Fewer Sexual Partners**- Having fewer sexual partners can reduce the chances of getting HPV and its types that can be of greater risks for development of the cervical cancer [82].

[2.5]Mevalonate pathway And FDFT1 -

Mevalonate pathway is one of the major types of metabolic pathway in which synthesis of lipids and sterol isoprenoids such as cholesterol and others such as non sterol-isoprenoids like dolichol or ubiquinone. But majorly this pathway is considered as a hallmark for the study of cholesterol biosynthesis and many studies are being done on it for its implications that can relate to pathological benefits [85].MVA pathway or the mevalonate pathway that uses Acetyl-CoA to produce isoprenoids and steroids that are related to the formation and growth of the tumor as well as its progression. The sterols produced by MVA pathway are essential for cancer progression. The genes that regulate the expression of MVA pathway enzymes are controlled by SREBP of transcription factor. In case of cancer cells the oncogenic alteration leads to uncontrolled expression of MVA pathway enzymes. Due to this unregulated pathway expression the cancer cells tend to get in extensive progression leading to proliferation and rapid multiplication [86].

MVA or Mevalonate is being synthesized from 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) by HMG-CoA reductase (HMG-CoAR) enzyme, which is a rate limiting agent for the pathway. MVA is further broken down into (FPP) Farnesyl Pyrophospate and Geranayleranyl Pyrophospate (GPP). These later play a major role in the protein prenylation which later tends to affect the cellular functions such as growth, division and proliferation. Many studies show that MVA pathway is upregulated in many cancers such as leukemia, lymphoma, multiple myeloma; as well as breast, hepatic, pancreatic, esophageal and prostate cancers. Several reasons are there which interrelates this deregulation of the MVA pathway that leads to progression cancerous tumors such as, p53 mutation, a mutation in HMG-CoAR and (SREBP) cleavage-activating protein SCAP as its regulator, PKB/Akt activation, decreased AMPK activation, and activation of transcription factors such as: SREBP and HIF-1 are some responsible factors out of multiple .Along with these many small guanosine triphospate hydrolases such as RHO and RAS also participate in the process of tumorgenesis due to their isoprenylation ones [87-90].

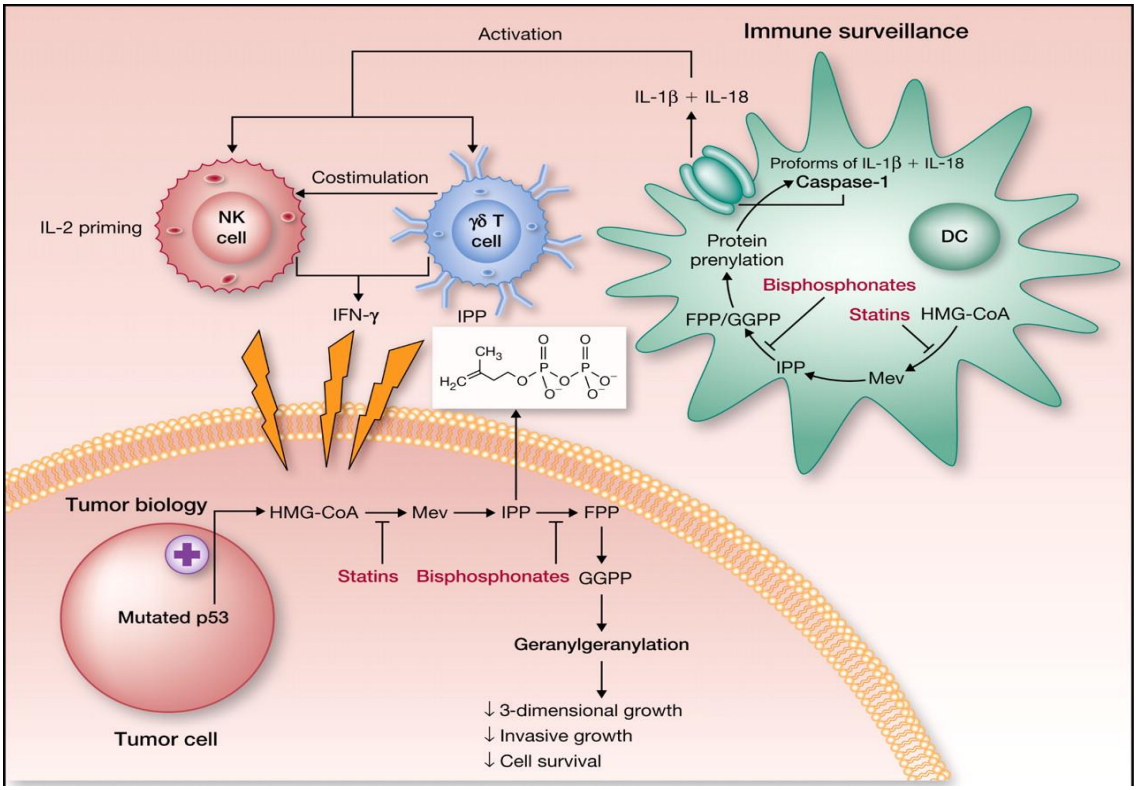


Figure 7: Effects of mevalonate pathway inhibitors on tumor biology and immune surveillance; Ref- Thurnher, Martin, Oliver Nussbaumer, and Georg Gruenbacher. "Novel aspects of mevalonate pathway inhibitors as antitumor agents." *Clinical Cancer Research* 18, no. 13 (2012): 3524-3531.

FDFT1- The result of mevalonate pathway or bio synthesis of cholesterol can be differentiated by the production of two byproducts, i.e., FPP and GPP. FPP or farnesyl pyrophosphate converts into squalene by the enzyme squalene synthase (SQS). The gene that encodes for the enzyme SQS is FDFT1 or Farnesyl disphospate Farnesyl transferase 1 gene, which is situated on the 8th chromosome at a position of 8p23. The net product size of this gene will be around 47kDA and it plays an important role in the MVA pathway since it is one of the regulatory gene of one of the enzyme in that pathway. The FDFT1 gene is mainly expressed in the liver and hypothalamus despite of its regular expression in the whole body. The gene promoter of FDFT1 has many sterol regulatory elements and it also has many isoforms with 8 exons mostly out of them [91,92].

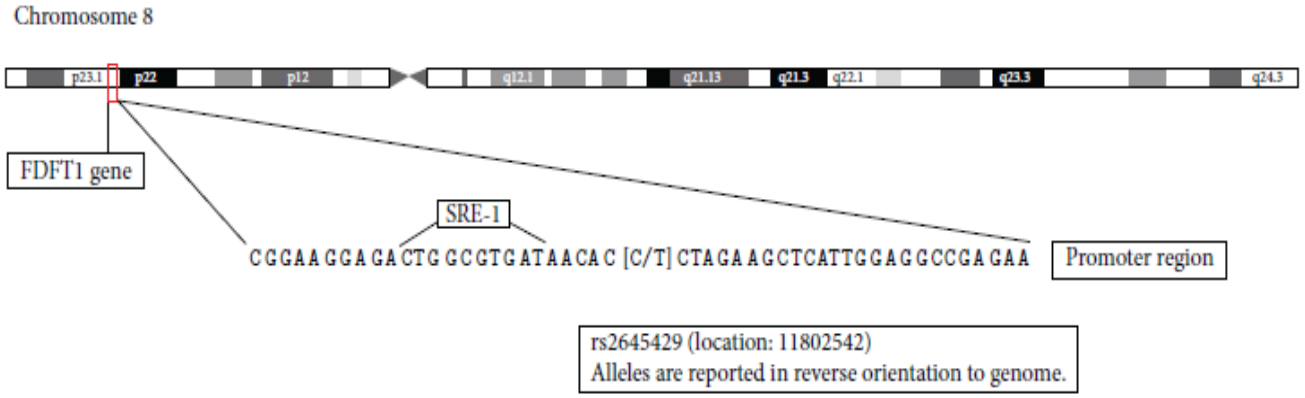


FIGURE 1: Location of the rs2645429 promoter SNP in the FDFT1 gene.

Figure 8: Location of rs2645429 promoter SNP in the FDFT1 gene; Ref-Dehghani, Mehdi, Zahra Samani, Hassan Abidi, Leila Manzouri, Reza Mahmoudi, Saeed Hosseini Teshnizi, and Mohsen Nikseresht. "Relationship of SNP rs2645429 in Farnesyl-Diphosphate Farnesyltransferase 1 Gene Promoter with Susceptibility to Lung Cancer." *International journal of genomics* 2018 (2018).

The role of FDFT1 has also been studied for checking the genetic diversity in other many diseases such as Hepatitis C, Cancers, etc. While the study of SNP rs2645429 polymorphism of the FDFT1 gene in Hepatitis C, it was being observed that this SNP was associated with the fibrosis of non fatty liver in patients [93]. The rs2645429 SNP of gene FDFT1 is situated at 6bp upstream of the putative SRE-1 and it has been studied that promoter activity is affected by replacement of one base on this polymorphism [94].

By checking and mentioning the above points in this study we will be looking for the relationship of rs2645429 FDFT1 and different common cancer like lung cancer, Head and Neck cancer and Cervical cancer for the first time on Indian population from Himachal Pradesh.

[2.6]SNP (Single Nucleotide Polymorphism)-

Single nucleotide polymorphism is a type of genetic variation that occurs at a specific position in the whole genome of a particular nucleotide as its substitution. SNPs occur once in a number of population which leads to certain genetic variation in that individual of particular species. This variation occurs due to genetic evolution or maybe because of mutation.

Through SNPs the susceptibility of genetic relations with many diseases have been find and still extensive research is still going on in this field in order to know th possible chances of mutation which is responsible for causing particular genetic diseases such as sickle cell anemia or Alzheimer's disease, etc.

SNPs can act as biomarkers that can help in defining the pattern that exists in the genome of population from a particular geographical region or from ethnicity, and their susceptibility to a particular disease. These studies about the SNPs can further help in checking for the possible treatments and solution in order to eradicate harmful genetically associated disease. For cancer there are many studies that show the possible mutations that can relate to a populations' susceptibility to get the disease. Other applications of SNP study can include its benefits in the roots of new fields such as, Forensics, Biomedical researches and pharmacogenetics [95,96].

On the basis of those studies here we are going to discuss the SNP rs2645429 for checking its relationship to common cancer among populations.

SNP Under study- rs2645429

Locus- 8p23

[2.7]PCR-RFLP-

In order to perform genotyping and find out the susceptibility of FDFT1 gene in the population and its relationship with common cancer among the people, RFLP can be used. Restriction Fragment Length Polymorphism or RFLP is a technique that is used to find out the differences and distinctiveness in a given DNA sequence which is often known as polymorphism. These differentiating patterns are used to find difference between different organism and these variable patterns are known as Variable Number of Tandem Repeats (VNTRs).

RFLP can be used to differentiate between the organisms interspecies and intraspecies. It was invented in 1984 by an English scientist, Alec Jeffreys during his study on hereditary analysis. Restriction endonucleases are enzymes that cut the DNA at particular sites known as restriction sites. Since the genetic variations are different in variant organisms and species, so the length of the fragmented DNA will differ accordingly. So in order to overcome that, the DNA fragments are being amplified using varied and specific primer set. To perform a PCR- RFLP there is a series of 4 steps, that can be the designing of the primer set that we will be using for the amplification of DNA up to particular size , so that the restriction endonuclease can cleave the DNA fragments at same positions . Second step will be the DNA isolation from the blood sample of the case person. Following to that next step will be the amplification of the DNA fragments using primers. After that amplified DNA is reacted with the restriction nuclease and finally they are visualized on the agarose gel using gel electrophoresis. Major applications of RFLP include the finding of genetic mappings to identify the recombination rates that show distance between genetic loci. Another major application can be in forensics and for the paternal identification [97].

[3]Experimental Objectives

There are 2 major objectives of this study-

1. Optimization of PCR-RFLP conditions for the chosen SNP rs2645429 using gradient PCR and multiple temperature range.

2. Analyzing the association of the SNP rs2645429 with FDFT-1 gene and its susceptibility with common cancers in the population of Himachal Pradesh.

[4]Materials And Methods

Population Study-

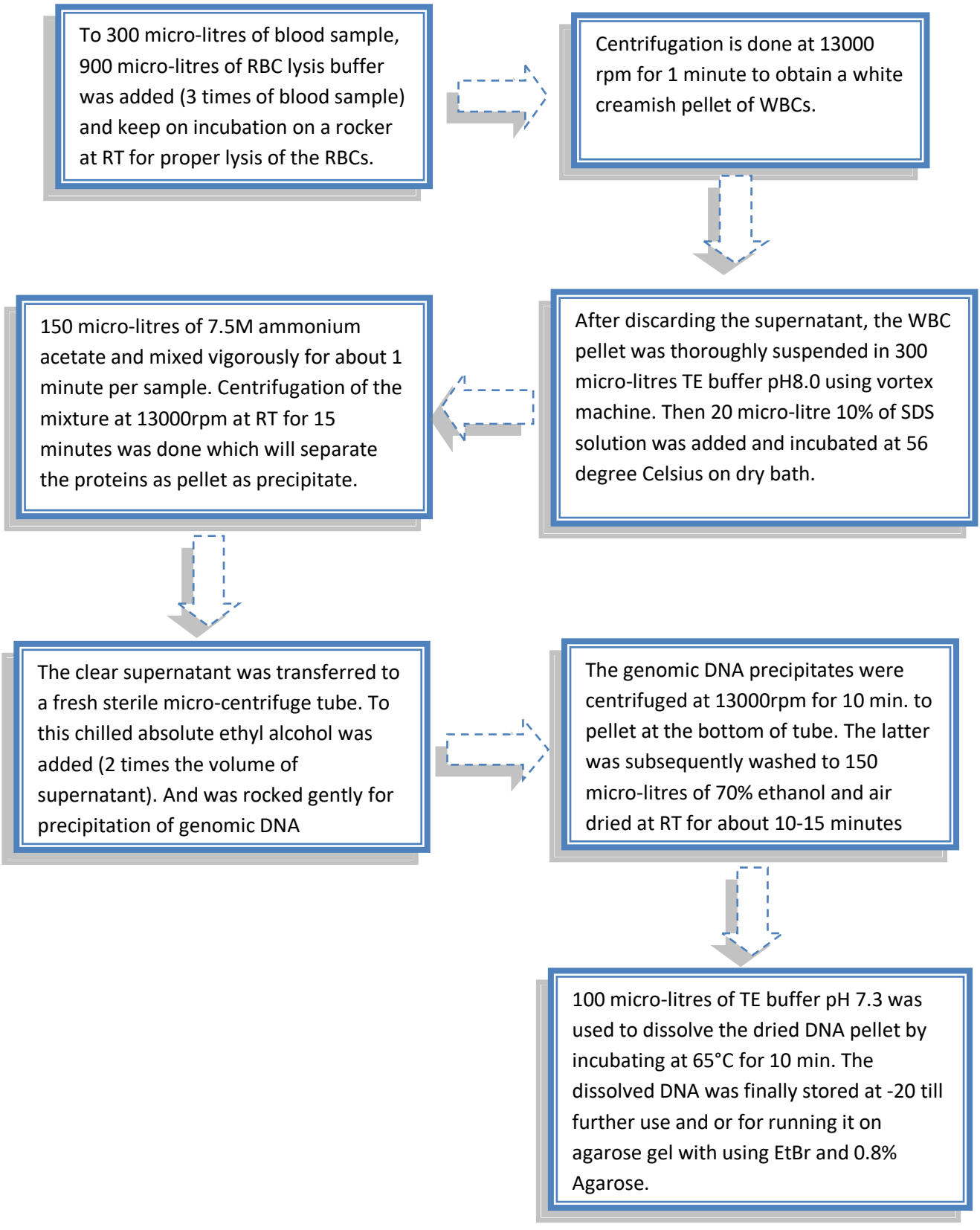
The study that we are performing here is based on the population of the Himachal Pradesh. A total number of around 395 samples of the cancer along with around 212 samples as control samples and most of them match according to the age and the geographical distribution around the state of H.P. Since for the study their DNA is required to be isolated and so it was performed after proper certifications and consent of the patient. Only after which the blood samples were drawn from the individual patient and labeled accordingly.

Sampling-

Sampling was done under proper supervision and with all necessary consent signed by the patient. Blood was drawn by sterilized needles and an amount of around 5 ml blood was taken from each patient which were then stored in EDTA coated vials and then placed in the cold storage at temperature lesser than -20°C in order to avoid degradation of DNA and clotting of the blood.

Isolation of Genomic DNA-

From the collected blood samples the DNA was being isolated using the salting out method and then stored in separate Vials in -20°C temperature. The following flowchart depicts various steps included in the process of DNA isolation by salting out method.



Steps to Isolate DNA from Blood Sample using the inorganic method or Miller Method

Primers-

For the further proceeding of the experimentation major step after the DNA isolation will be amplification but for that primers are required to be designed. Here in our study we retrieved the primers from the reference study that our whole study is based one. After the retrieval the following primers were also re confirmed using the NCBI BLAST which gave the full confirmation for the same.

Forward Primer-

5′-GCTGGACCTGTGGAGTAGGT-3′

Reverse Primer-

5′-CTCCTGCGCATCCTAAGC-3

After the confirmation these set of primers were ordered using university resources and further the PCR-RFLP experimentation was proceeded ahead [98].

Reconstitution of Primers-

After the primers were received, they were supposed to be reconstituted and dilutions were made out of the control samples whose concentrations are optimized up to 100µM. For doing the same following amount of nuclease free water was added to the lyophilized primers.

Table 1: Reconstitution of primers

Primers	NFW (µL)
Rs2645429 Forward	281.8
Rs2645429 Reverse	325.7

PCR Optimization-

For finding out the most effective annealing temperature for the reconstituted primer set, Gradient PCR were run using the defined temperature range and then checked. With optimized temperature for the primers the further amplification process will be much more efficient.

PCR-

The gradient for the temperature optimization and the DNA amplification was done using PCR or Polymerase Chain Reaction. Gradient PCR were done using a range of 6 different temperatures at a time for the optimization.

Following PCR reaction mixture was used for setting up a reaction of 12µL per reaction.

Table 2: Reaction volume of constituents of PCR reaction

Reaction Components	Volume per Reaction (µL)
Master Mix	5
DNA Template	1
Forward Primer	0.5
Reverse Primer	0.5
Nuclease Free Water	5
Total	12

PCR Cycling conditions set for Gradient –

Table 3: Reaction conditions and cycles for Gradient PCR

Steps	Temperature (°C)	Time	Cycle/s
Initial Denaturation	95	3 minutes	1
Denaturation	95	35 seconds	
Temperature Range	50,52,54,56,58,60	35 seconds	35 cycles
Extension	72	1 minute	
Final Extension	72	5 minutes	1
Hold	4	∞	-

After the gradient PCR the optimized temperature was found out to be 56°C.

Genotyping method for PCR-RFLP –

After the annealing temperature was optimized at 56°C , the PCR was performed and later their genotyping was done. After PCR amplification of DNA, the result was visualized on 2% agarose gel using the suitable amount of illuminiscence dye such as EtBr (Ethidium Bromide).

Table 4: Reaction conditions and cycles for PCR amplification

Steps	Temperature (°C)	Time	Cycle/s
Initial Denaturation	95	3 minutes	1
Denaturation	95	35 seconds	35 cycles
Temperature Range	56	35 seconds	
Extension	72	1 minute	
Final Extension	72	5 minutes	1
Hold	4	∞	-

After the amplification of the DNA genotyping of the samples was done by performing restriction digestion using enzyme XbaI and placed for incubation overnight and then again visualized on the 2% agarose gel electrophoresis.

Analysis of Digested Product-

After putting the PCR product under digestion by XbaI, the final resultant products were visualized on the agarose gel and the banding pattern was observed under UV gel dock for the recording of the observations. The banding pattern will tell the resultant genotype of the digested product.

Table 5: Reaction mixture and amount for restriction digestion

Reaction Components	Reaction Volume (µL)
Restriction enzyme (1 Unit)	0.1
Buffer	1.5
Nuclease Free Water	3.5
DNA	10
Total	15

Statistical Analysis-

After getting the whole observation and performing genotyping on over around 600 samples, the statistical analysis is required for assessing the association of FDFT1 gene polymorphism with cancer susceptibility. The genotype frequency can be calculated from Hardy Weinberg equation and using its calculation tool present online. The observed frequency is mainly to understand the frequency of disequilibrium which is a case when the value of p should be <0.05. In order to assess the risk association the odds ratio and the 95% CI is to be calculated using "Medcalc" tool after which its significance can be checked by Z-value.

Table 6: Size of banding pattern for different genotypes

For rs2645429 banding patterns and genotype	
Genotype	Band Size (Base Pairs)
CC (Wild Type Homozygous)	376
CT (Heterozygous)	376+224+152
TT (Mutant Type Homozygous)	224+152

[5]Observations and Results

Fort the Temperature optimization of the Primers the following observations were taken from the Gradient PCR.

The following picture shows the gradient over range of 6 different temperatures-

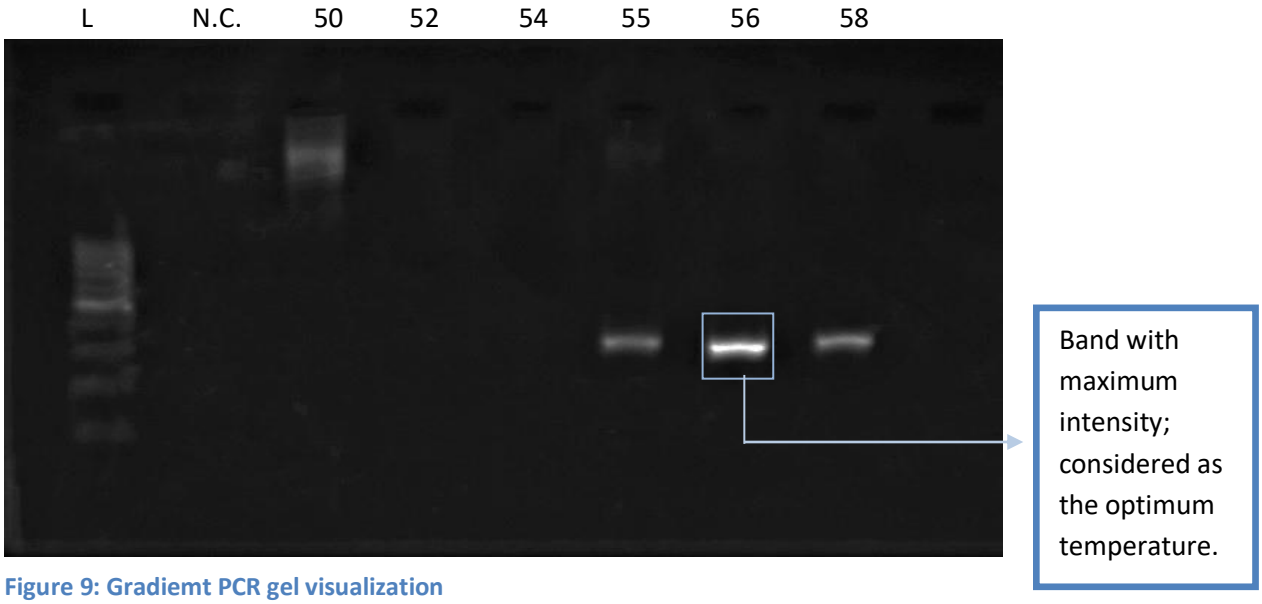


Figure 9: Gradiemt PCR gel visualization

Depiction-

Lane 1: Ladder 100 bp; Lane2: Negative Control; Lane 3: 50°C; Lane4: 52°C;

Lane 5: 54°C; Lane 6: 55°C: Lane7: 56°C; Lane 8: 58°C.

From the above gel picture it can be depicted that at 56°C, the intensity is the maximum and the PCR product is been amplified to its maximum from the sample template DNA that we used.

PCR amplification –

From the following observations we can see the amplified PCR products that were to be digested further for analyses.

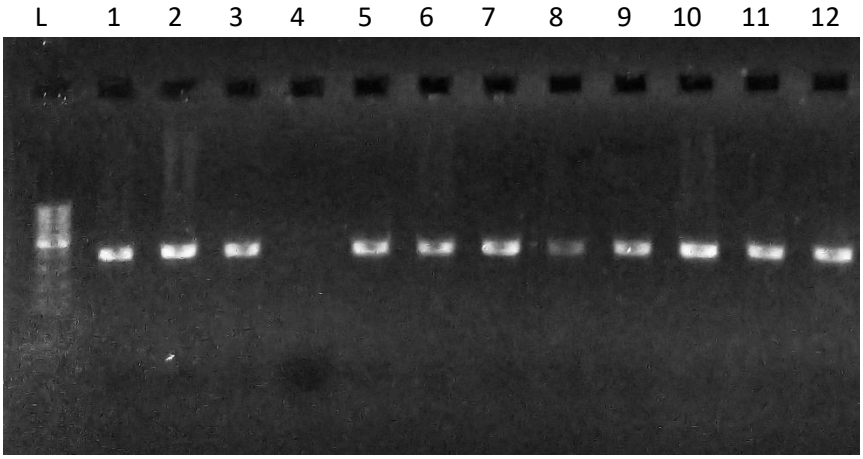


Figure 10: PCR amplification gel visualization

Depiction:

Lane1- Ladder; Lane2- Amplified PCR Product; Lane3- Amplified PCR Product; Lane4- Empty (No amplification); Lane4- Amplified PCR Product; Lane6- Amplified PCR Product; Lane7- Amplified PCR Product; Lane8- Amplified PCR Product; Lane9- Amplified PCR Product; Lane10- Amplified PCR Product; Lane11- Amplified PCR Product; Lane12- Amplified PCR Product.

From the above taken observations of the amplified DNA product from PCR it can be concluded that the annealing temperature that has been derived from the Gradient PCR worked correctly and very efficiently.

Genotyping Results-

After the successful PCR amplification, the next step will be the Restriction Digestion of the product to analyze the banding pattern for the presence of mutant or heterozygosity in the phenotype.

Following Image depicts the various patterns after the genotyping-

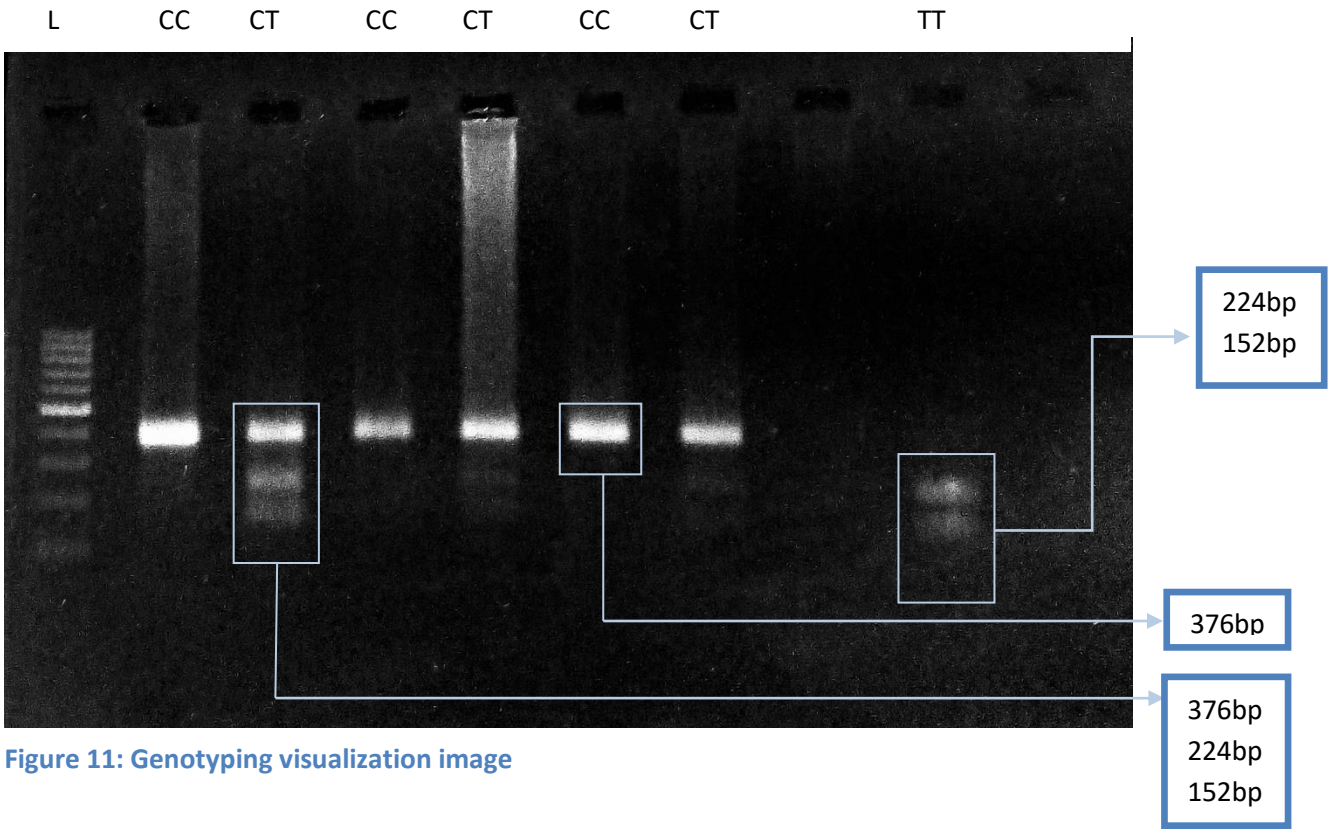


Figure 11: Genotyping visualization image

Depiction-

Lane 1: Ladder (100bp); Lane 2: CC (Homozygous); Lane3: CT (Heterozygous); Lane4: CC (Homozygous); Lane5 : CT (Heterozygous) ; Lane6: CC (Homozygous); Lane7 : (Blank); Lane8: TT (Mutant).

The above mentioned observations are depicting the different banding pattern of the wild type homozygous, the heterozygous and the complete mutant type genotype in a given population.

[6]Discussion

A total of 173 viable control samples and along with them 131 case samples of Head and neck cancer , 122 case samples of lung cancer and 89 case samples of cervical cancer were analyzed for rs2645429 in the gene FDFT1. After the genotyping of FDFT1 gene it was found that the T allele was present in 8% of the cases of head and neck cancer and among 15% of control cases. Similarly in case of lung cancer T allele was present in 28.6% of the case samples and 15% in the control case, and for cervical cancer the frequency for case control is 26% and for the control sample the same was 15%. For rs2645429-

Table7: Analyzing the data for presence of T allele in cervical cancer

Genotype/ Allele	CX (n/N[%])	Control (n/N[%])	OR	CI	p-value
CC	68/89 (76.4%)	146/173(84.3%)		Ref	
CT	21/89 (23.5%)	27/173(23.5%)	1.6699	0.8816 to 3.1631	0.1156
TT	0/89 (0%)	0/173 (0%)	2.1533	0.0423 to 109.6678	0.7046
CC+TT	21/89(23.5%)	27/173(23.5%)	1.6699	0.8816 to 3.1631	0.1156
C	89/89 (100%)	173/173 (100%)		Ref	
T	21/89 (23.5%)	27/173 (15.6%)	1.6699	0.8816 to 3.1631	0.1156

Table8: Analyzing the data for presence of T allele in head and neck cancer

Genotype/ Allele	HNC (n/N[%])	Control (n/N[%])	OR	CI	p-value
CC	120/131 (90.8%)	146/173		Ref	
CT	11/131 (8.3%)	27/173	0.4957	0.2361 to 1.0405	0.0636
TT	0/131 (0%)	0/173	1.2158	0.0239 to 61.7306	0.9223
CT+TT	11/131(8.3%)	27/173	0.4957	0.2361 to 1.0405	0.0636
C	131/131 (100%)	173/173		Ref	
T	11/131 (8.3%)	27/173	0.4957	0.2361 to 1.0405	0.0636

Table9: Analyzing the data for presence of T allele in lung cancer

Genotype/ Allele	LC (n/N[%])	Control (n/N[%])	OR	CI	p-value
CC	83/122 (68%)	146/173 (84.3%)		Ref	
CT	38/122 (31.1%)	27/173 (23.5%)	2.4462	1.3950 to 4.2895	0.0018
TT	1/122 (0.7%)	0/173 (0%)	5.2012	0.2095 to 129.1186	0.3143
CT+TT	39/122(31.9%)	27/173(23.5%)	2.5106	1.4351 to 4.3922	0.0013
C	121/122(99.1%)	173/173(100%)	1.4578	1.0156 to 2.0926	0.0410
T	39/122 (31.9%)	27/173 (23.5%)	2.5106	1.4351 to 4.3922	0.0013

So from the above observations we can conclude that, the allelic frequencies as calculated from the HWE for the different cancers for SNP rs2645429 where "p" is frequency of wild type allele and "q" is frequency for mutant type were-

For HNC p=0.958; q=0.042, for LC p=0.8361; q=0.1639, for CX p=0.882; q=0.118.

Since only one mutant was found that too in the case samples of the lung cancer so after calculation the carrier frequency of lung cancer here was found out to be 1 in 6.07 i.e., around 16.47% among all the case sample population.

And the resultant statistical analysis as done with the help of the Medcalc for rs2645429 for different cancers is listed as- For Cervical cancer: for (CT) OR=1.6699, p=0.1156, CI=0.8816 to 3.1631; for (TT) OR=2.1533, p=0.7046, CI=0.0423 to 109.6678.

For Head and Neck cancer: for (CT) OR=0.4957, p=0.0636, CI=0.2361 to 1.0405; for (TT) OR=1.2158, p=0.9223, CI=0.0239 to 61.7306.

For Lung Cancer: for (CT) OR=2.4462, p=0.0018, CI=1.3950 to 4.2895; for (TT) OR=5.2012, p=0.3143, CI=0.2095 to 129.1186; for (T) OR=2.5106, p=0.0013, CI=1.4351 to 4.3922; for (C) OR=1.4578, p=0.0410, CI=1.0156 to 2.0926.

After the analysis it was observed that the allele "T" is having association among the population for lung cancer for the SNP rs2645429. Hence, it can be concluded that whoever will have this mutant allele in their genotype will have greater chances of having the disease.

[7]Conclusion and Future Prospects

After performing the genotyping in association with the SNP rs2645429 to check its susceptibility with association to common cancers in Himachal Pradesh population, the results show that only lung cancer has its association of variant rs2645429 with the population of Himachal Pradesh, and only the mutant allele for lung cancer has its association for the SNP rs2645429 and not with the Head and Neck cancer or with the cervical cancer. This data can further be used to check the range of mutation that the population of Himachal Pradesh has. Further this can be compiled in order to check the susceptibility with population of other states or cities in India and check the pattern of mutation.

Appendix

1) GLASSWARE AND INSTRUMENTS

Glassware

- Beaker – 1000 ml, 500 ml, 100 ml
- Microcentrifuge tubes – 2 ml, 1.5 ml, 0.5 ml and 0.2 ml
- Measuring cylinder – 500 ml, 100 ml
- Autoclaved microtips (100 -1000 µl; 20 – 200 µl; 0.1 – 10 µl)
- Capped Bottles
- PCR tube stand

Instruments Used-

- Micro pipette
- Thermo-Cycler PCR machine
- Laminar Air Flow Hood
- Autoclave
- Incubator
- Hot air oven
- pH meter
- Microwave Oven
- Centrifuge
- Water Bath

REAGENTS

Di-sodium ethylene diamine tetra acetate, Na_2EDTA (0.5M, pH 8.0)

18.61g of Na_2EDTA was dissolved in 50ml MQ by magnetic stirring. And Simultaneously and dropwise 10M NaOH was added till pH 8.0 was reached, and final volume was raised to 100ml after dissolving the salt completely in the solution.

Tris (hydroxylmethyl) aminomethane-chloride, Tris-Cl (1M, pH 8.0)

12.11g of Tris Base was dissolved in 75ml of sterile MQ water. And pH was set at 8.0 using 1N HCl. Volume was raised to 100 ml with MQ water. The resulting product was filtered using Whattmann filter paper and stored in a sterile screw capped reagent bottle.

Tris (hydroxylmethyl) aminomethane-chloride, Tris-Cl (1 M, pH 7.3)

12.11g was dissolved Tris Base in 75ml of sterile MQ water. And the pH was set at 7.3 using 1N of HCl and the volume was raised to 100 ml with MQ water. The resulting product was filtered using Whattmann filter paper and then stored in a sterile capped reagent bottle.

Ammonium Chloride, NH_4Cl (1M)

5.35g of Ammonium Chloride was dissolved in 80ml MQ water. The final volume was raised to 100 ml.

Sodium dodecyl sulphate, SDS (10%)

10gm of SDS was mixed in 70ml of MQ water. Volume raised 100ml.

RBC lysis Buffer

10mM Tris-Cl(1 M, pH 8.0) + 1mM EDTA + 125mM NH_4Cl (pH 8.0)

Mixed:

1M Tris-Cl, pH 8.0	10 ml
0.5M EDTA	2 ml
1M NH_4Cl	125 ml

Volume was raised up to 1000ml by MQ water.

Tris EDTA (TE) Buffer: pH 8.0

Mixed:

1M Tris Cl; pH 8.0	10 ml
0.5M EDTA	2 ml

Volume raised to 1000 ml by MQ water.

Tris EDTA (TE) Buffer: pH 7.3

Mixed:

1M Tris Cl; pH 7.3	10 ml
0.5M EDTA	2 ml

Volume raised to 1000 Buffer ml by MQ water.

Ammonium acetate (7.5M)

28.9g of Ammonium acetate was dissolved in 20 ml MQ water and the final volume was raised to 50ml.

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