INTRODUCTION

Telomeres are DNA–protein complexes that cap chromosomal ends, promoting chromosomal stability. When cells divide, the telomere is not fully replicated because of limitations of the DNA polymerases in completing the replication of the ends of the linear molecules, leading to telomere shortening with every replication ***(Chan and Blackburn, 2004).*** To protect the germ line and the subpopulation of stem cells from senescence, mechanisms have evolved to prevent telomere attrition in these cellular compartments. The most common and best studied mechanism involves the activation of telomerase. Telomerase is a ribonucleo-protein enzyme with reverse transcriptase activity that is capable of extending chromosomal ends with specific telomeric DNA sequences by using a portion of its RNA component as a template ***(Forsyth et al., 2002; Tomas-Loba et al., 2008).*** Thus Telomerase has direct telomere protective function ***(Chan and Blackburn, 2003).*** Healthy lifestyle changes (*e.g.*, improved diet and increased exercise) were associated with a significant increase in telomerase activity and longer telomeres ***(Bekaert et al., 2007; Ornish et al., 2008).***

The telomerase enzyme is normally inactive in most somatic cells, with the exception of proliferative stem cells, germ line cells, and activated lymphocytes. Telomerase activity is highly expressed in most malignant tumors, accounting for

hyperproliferation and resistance to apoptosis. & it may also be responsible in part for some non malignant proliferative skin diseases including psoriasis. Also increased telomerase activity and shortened telomere length has been reported in T cells of blood of patients with atopic dermatitis and psoriasis indicating that T lymphocytes in atopic dermatitis and psoriasis are chronically stimulated and have an increased cellular turnover) ***(Kaida et al., 2000; Moushira et al., 2006; X.Liu et al., 2008).***

Telomere shortening has been considered an aging marker since it represents lifetime exposure to oxidative and inflammatory stress, and it has been shown to be negatively correlated with age, smoking, and mortality. Skin aging is a continuous process that affects skin function and appearance. However, not everybody ages at the same speed. Intrinsic, environmental, and lifestyle factors contribute to the pace of skin aging ***(Lavker, 1995; Yaar et al., 1998; Aviv, 2004).***

There are two types of aging, intrinsic aging and extrinsic aging. Intrinsic aging is the slow irreversible degeneration of tissue that affects almost all body organs. Extrinsic factors are, to varying degrees, controllable and include exposure to sunlight, pollution or nicotine, and miscellaneous lifestyle components such as diet and overall health ***(Farage et al., 2008; Datta et al., 2009).***

The harmful effects of chronic sun exposure (photoaging) and smoking on premature skin aging are widely

supported. Cigarette smoking is a well-established independent risk factor for facial wrinkling and skin aging. Cigarette smoking is strongly associated with elastosis, and telangiectasia. Smoking causes skin damage primarily by decreasing capillary blood flow to the skin, which, in turn, creates oxygen and nutrient deprivation in cutaneous tissues. It has been shown that those who smoke have fewer collagen and elastin fibres in the dermis, which causes skin to become slack, hardened and less elastic. A clear dose–response relationship between wrinkling and smoking has been identified, with smoking being a greater contributor to facial wrinkling than even sun exposure ***(Kennedy et al., 2003; Rexbye et al., 2006; Farage et al., 2008).***

Tobacco smoke extract alters the function of human skin fibroblasts and affects the extracellular matrix (ECM) turnover in vitro. The exposure of human skin fibroblasts to tobacco smoke extract has been shown to decrease biosynthesis of type I and III collagens. In addition, significantly higher levels of matrix metalloproteinase-1 were observed in non sun exposed skin of smokers compared to non smokers. Decreased telomere length has been associated with smoking ***(Yin et al., 2000***; ***Lahman et al., 2001; Morlà et al., 2006; McGrath et al., 2007; Aviv et al., 2009)***.

Thus, Smoking status, diet, socioeconomic status, stress level, and lifestyle might influence telomere dynamics ***(Getliffe et al., 2005; Mirabello et al., 2009; Woo et al., 2009).***

AIM OF THE WORK

To study human telomerase reverse transcriptase expression in skin of smokers and non smokers, to investigate the possible role of the telomerase in the pathogenesis of aging and the lifestyle factors that may affect telomerase, human health and aging.

SMOKING

Smoking is a practice in which a substance, most commonly tobacco less common **cannabis**, is burned and the smoke is tasted or inhaled. This is primarily practiced as a route of administration for recreational drug use, as combustion releases the active substances in drugs such as nicotine and makes them available for absorption through the lungs. The most common method of smoking today is through cigarettes, primarily industrially manufactured but also hand-rolled from loose tobacco and rolling paper. Other smoking implements include pipes, cigars, bidis, hookahs, vaporizers and bongs ***(Saha, 2009).***

Tobacco use is the leading preventable agent of death in the world. It is responsible for more than five million deaths each year & the death toll from tobacco is expected to climb to > 8 million people per year within next 25 years. It is estimated that eventually 50% of all smokers will be killed by direct or indirect effects of tobacco. Use of tobacco is also increasing esp. in developing countries, in teenagers & in women. As in 2002, some 1.22 billion people smoked. It was predicted that by 2010, 1.45 billion people will smoke and 1.5 to 1.9 billion by 2025 ***(CDC 2008; Saha, 2009).***

1-1 History and consumption:

The history of smoking dates to 5000 BC. Early smoking evolved in association with religious ceremonies for purpose of spiritual enlightenment. The practice quickly spread from Europe & America to the rest of the world ***(Gilman and Xun, 2004).***

Tobacco **'**Brown gold', is an agro based product processed from fresh leaves of plants in genus "Nicotiana'. Of the several species; *Nicotiana tabacum* is commonly grown, but *Nicotiana rustica* also contains high concentrations of nicotine. The leaves are harvested, cured (slow oxidation and degradation of carotenoids in tobacco leaf)**,** is treated, mixed with additives and then pyrolyzed. Tobacco is combined with up to 599 additives to enhance the addictive potency, improve the effect & make it more palatable. The resulting vapors are inhaled and active substances absorbed through lung ***(Lioyd and Mitchinson, 2006).***

Tobacco has Nicotine (2-5%, +/- 0.23%), Sugars (mainly reduced) (8-25%, +/- 1.8%) and Moisture (10-14% +/- 0.3%).

The word 'Nicotine' is derived from Frenchman Jean Nicot who introduced tobacco to France in 1560 It is consumed in two forms, as:

1. Smoke
2. Smokeless

Smoke:

Tobacco for smoking is available as Cigar, Cigarettes, Electronic cigarette (provides nicotine vapor from nicotine solution), and Pipe. Vaporizer is used to sublimate the active ingredient in partial vacuum, rather than burning, with less production of irritating, toxic, carcinogenic by-products. Each cigarette can cause much damage (Table 1) **(Saha, 2009)**

Table (1): Facts about one cigarette

• On average, each cigarette shortens a smoker's life by around 11 minutes.
• A single cigarette can reduce the blood supply to skin for over an hour.
• Cigarettes contain more than 3500 chemical compounds, at least 60 are carcinogenic.
• When one inhales, a cigarette burns at 700°C at the tip and at 60°C in the core.
• The British Medical Association estimates that up to 120,000 men have ED because of smoking

Non smoking exposure (smokeless):

Even non smokers are not exempt from the adverse health effects of smoking. Because of its negative implications; this form of consumption has played a key role in regulation of tobacco products. Smokeless tobacco also contains nicotine. Passive smoking is involuntary consumption of tobacco smoke ***(Hecht, 2004)***.

1-2 Constituents of tobacco smoke:

The tobacco smoke can be separated into:

A. Particulate phase **B.** Gas phase

The gas phase (vapor phase) comprises over 90% of the mainstream smoke weight ***(Hoffmann et al., 2001).***

Tobacco smoke is an aerosol containing about 10^{10} particles/mL, consisting of highly porous carbonaceous polymeric material with adsorbed heavy metals, polycyclic aromatic hydrocarbons (PAH), azaarenes, N-nitrosamines and various other organic chemicals ***(Hecht, 1999).***

The main constituents of the vapor phase are nitrogen, oxygen, and carbon dioxide. Potentially carcinogenic vapor phase compounds include nitrogen oxides, isoprene, butadiene, benzene, styrene, formaldehyde, acetaldehyde, acrolein, and furan ***(Hoffmann et al., 2001)***.

The particular phase of tobacco smoke contains at least 3,500 chemical compounds and a high proportion of them are toxic, carcinogens or mutagens, (e.g. benzene, 2-napthylamine, ^{210}Po, ^{226}Ra, ^{228}Ra, nickel, cadmium, benzo[a]pyrene, etc) ***(Hoffman, 1997; Hecht, 1999).***

The carcinogens in tobacco smoke:

There are over 60 carcinogens in cigarette smoke that have been evaluated by the International Agency for Research on Cancer, they belong to various classes of chemicals, as follows:

PAH (10 compounds), aza-arenes (3), *N*-nitrosamines (8), aromatic amines (4), heterocyclic amines (8), aldehydes (2), volatile hydrocarbons (4), nitro compounds (3), miscellaneous organic compounds (12), and metals and other inorganic compounds (9). Other carcinogens not evaluated by IARC are also likely to be present. For example, among the PAH, multiple alkylated and high molecular-weight compounds have been detected ***(Hoffmann et al., 2001).***

Assessment of smoking severity:

Smoking index (SI) is defined as the product of multiplying the number of cigarettes smoked per day by the numbers of years of smoking

Categories *include the following:*

Mild: Those having (SI) less than 200

Moderate: Those having (SI) between 200-400

Heavy: Those having (SI) more than 400 ***(Borland et al., 2010)***

Tian et al (2011) subdivided Current smokers into light smokers (<20 cigarettes per day) and heavy smokers (≥20 cigarettes per day). Former smokers were also further subdivided into 2 subgroups by quitting time: long-term quitters were those who had quit at least 5 years earlier, and short-term quitters were those who had quit less than 5 years earlier. A never smoker was defined as a person who had never smoked or had only smoked infrequently at a young age.

1-3 Health morbidities caused by tobacco smoking:

Diseases associated with cigarette smoking:

1- 3-1: Cardiovascular system diseases: ***(Tsiara et al., 2003; Bullen, 2008; Winkelmann et al., 2010).***

- Atherosclerotic cardiovascular disease e.g. hypertension
- Coronary artery diseases, coronary artery spasm
- Carotid vascular diseases
- Vascular aneurysms
- Arrhythmias
- Vascular insufficiency e.g. ischemic and peripheral vascular diseases as burger's disease, deep venous thrombosis, pulmonary embolism

1-3-2: Neoplastic diseases: ***(Blackford et al., 2009; Stern et al., 2009; Burlakova et al., 2010).***

- Respiratory tract neoplasms e.g bronchogenic carcinoma
- Laryngeal cancer
- Oral cancer
- Leukemia
- Others e.g. cancer esophagus, pancreas, bladder, stomach.

1-3-3: Pulmonary diseases:

- Emphysema and COPD ***(Wang et al., 2010)***
- Asthma and bronchitis ***(Wagena et al., 2004)***
- Pneumothorax, Oesinophilic lung granuloma and suppurative lung syndrome ***(Murin et al., 2000)***
- Sleep apnea ***(Kashyap et al., 2001)***

1-3-4: Gastrointestinal diseases:

- Peptic ulcer and reflux eosophagitis ***(Parasher and Eastwood, 2000)***
- Chronic pancreatitis ***(Andriulli et al., 2010)***
- Crohn's disease ***(Van der Heide et al., 2009)***
- Colorectal cancer ***(Limsui et al., 2010)***
- Tobacco smoking is a risk factor for small bowel carcinoid tumor "SBC" ***(Kaerlev et al., 2002)***

1-3-5: Reproductive diseases*:*

- Impotence (due to vascular insufficiency) ***(Linnebur, 2006)***
- Spermatogenesis abnormalities ***(Kilic et al., 2009)***

1-3-6: Rheumatological diseases:

- Osteoporosis ***(Moinuddin et al., 2008)***
- Rheumatic arthritis ***(Sugiyama et al., 2010)***

1-3-7: Psychiatric diseases:

- Depression ***(Boden et al., 2010)***
- Schizophrenia ***(Tsoi et al., 2010)***

1-3-8: neurological diseases:

- Dementia ***(Rusanen et al., 2011)***
- Peripheral neuropathy ***(Carratù et al., 2008)***
- Cerebrovascular strokes ***(García-Núñezet al., 2007)***

1-3-9: Endocrinal diseases:

- Goiter ***(Ittermann et al., 2008)***

1-3-10: Renal diseases:

- Glomerulonephritis ***(Yacoub et al., 2011)***

1-3-11: Infectious diseases:

- Impaired immunity ***(Mehta et al., 2008)***

- Increased the incidence of various infections e.g. pneumococcal, and meningiococal ***(Huttunen et al., 2011)***

1-3-12: Haematological diseases:

- Smokers have elevated levels of carboxyhaemoglobin (COHb) in the blood ***(Cronenberger et al., 2008)***
- Oesinophilia ***(Petäys et al., 2003)***
- Increase risk of leukemia, myeloproliferative diseases and myelo dysplasia ***(Lichtman et al., 2007)***
- Increase blood viscosity and liability to thrombosis (***Pomp et al., 2008)***

1-3-13: Environmental hazards:

Passive or forced environmental tobacco smoking is the involuntary inhalation of tobacco smoke by non smokers; exposure to side stream was included as strong possible predisposing factor to COPD ***(Menezes and Hallal, 2007).***

1-4 Smoking induces oxidative stress and telomere shortening:

1-4-1 Smoking and oxidative stress:

The mechanisms responsible for changes occurring during skin aging processes are complex. This complexity is in part due to the fact that both intrinsic and extrinsic determinants are cumulated. Among extrinsic influences, it is well established that life style factors, such as cigarette smoking, and

environmental factors, such as sun exposure and pollution, contribute largely to the skin aging process. A common point between all these factors is their ability to induce ROS generation, leading to an oxidative stress that creates cellular damage, disruption in the redox status and loss of molecular functions ***(Kennedy et al., 2003)***.

Cigarette smoking was reported to increase oxidative DNA modification in humans. A link exists between oxidative DNA modification, accelerated aging and cancer. Earlier studies focused on cigarette smoking-induced DNA damage in the lung, however, it soon became obvious that systemic exposure to circulating cigarette smoke constituents results in an increased presence of elevated levels of DNA adducts in tissues not directly exposed to tobacco smoke ***(Loft et al., 1992; Csiszar et al., 2009).***

Exposure to cigarette smoke is the major cause of chronic oxidative stress in human ***(Muscat et al., 2004).*** It promotes oxidative stress by the direct delivery of ROS and by promoting the endogenous generation of ROS by activation of inflammatory cells ***(Burke and FitzGerald, 2003).*** Long-term smoke exposure can result in systemic oxidative stress as reflected by depleted levels of antioxidants. The systemic effects of cigarette smoke are mediated by its role in induction of systemic oxidative stress and inflammation ***(Yanbaeva et al., 2007)***.

Also, it has become clear that DNA repair processes have equal importance for accelerated aging. Repair of oxidative DNA damage is extensive and differences in DNA repair are proposed to be important for cancerogenesis and premature aging as well. There are a large number of enzyme systems that recognize oxidative DNA modifications and start a multistep process of repair. Long-lived species have superior DNA repair compared to related short-lived species ***(Csiszar et al., 2007)***. In humans many of the enzyme systems involved in DNA repair exhibit single nucleotide polymorphisms (SNPs), which are associated with an increased risk for cancer development. The relationship between cigarette smoke-induced oxidative DNA damage in different tissues and carcinogenesis are widely appreciated. An important hypothesis also suggest a direct link between oxidative DNA modification and the aging process ***(Shigenaga et al., 1994; Csiszar et al., 2009)***

Cigarette smoke produces a high load of reactive oxygen species (ROS). Consequently, the oxidant/antioxidant balance in smokers becomes disturbed and leads to oxidative stress. Oxidative stress damages mitochondrial and nuclear DNA, proteins, as well as lipids ***(Huang et al., 2010).***

However, human beings have developed several defences against environmental attacks. The first one is the physical barrier constituted by the skin that protects the organism against UV penetration. Intracellular physiological enzymatic and non-enzymatic antioxidants also participate in