Study of Genetic Susceptibility to Oxidative Stress and DNA Damage Repair Gene in Male Brick Kiln Workers.

BY

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QUAID-I-AZAM UNIVERSITY,

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A dissertation submitted in the partial fulfilment of the requirements for the degree of

MASTER OF PHILOSOPHY IN ZOOLOGY

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Author's Declaration

I hereby affirm that the work presented in the following thesis is my individual effort, and all the content within this thesis is of my own creation. I have not previously submitted any portion of this work for consideration towards any other academic degree or elsewhere.

Zainab Kalsoom

List of Abbreviations

Abbreviation	Full Form
BER pathway	Base excision repair pathway
Cys	Cysteine (amino acid)
(C>G)	(Cytosine > Guanine)
EDTA	Ethylenediamine tetracetic acid
hOGG1	8-Oxo-Guanine Glycosylase 1
LIGIII	Ligase III
8-oxoG	8-Oxo-Guanine
Pol β	Polymerase β
PCR	Polymerase chain reaction
PAHs	Poly-cyclic aromatic hydrocarbons
ROS	Reactive oxygen specie
SPM	Suspended particulate matter
Ser	Serine (amino acid)
TE buffer	Tris EDTA buffer

Acknowledgement

All praises to Almighty Allah the Creator of the heavens and the earth, the Giver of life and sustenance. His boundless mercy, wisdom, and guidance have been a constant source of strength and inspiration throughout my journey. I am profoundly thankful for His blessings, protection, and the countless opportunities He has bestowed on me. I extend my utmost respect and gratitude to Holy Prophet Hazrat Muhammad (SAWW), the beloved messenger of Almighty Allah and the embodiment of divine guidance. I am indebted to Him for bringing us out of darkness and enlightening the way of heaven.

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Zainab Kalsoom

1: Abstract

A DNA damaging repair gene, 8-Oxo-Guanine Glycosylase 1 *(hOGG1)*, encodes glycosylase enzyme with involvement of inflammatory physiological pathways, plays crucial roles in protecting the body from cancer progression and oxidative pathologies. DNA glycosylases fix the DNA damage and remove oxidized base (8-Oxo-G) through a mechanism called base excision repair pathway (BER). The repair action of glycosylases is highly sensitive to heavy metals because they have an ability to bind sulphur group of cysteine residues and form dimer shapes to destabilize the original configuration of DNA and proteins. Literature has reported that variations in exon 7, especially transversion of C>G at codon 326 of *hOGG1* gene, is responsible for the inactivation of DNA glycosylase repair activity. Heavy metals are one of the major factors for occurrence of DNA base transversions and genetic polymorphism. The brick kiln industry releases heavy metals at appreciably higher concentration. Number of studies reported that heavy metals from the brick kiln industry induces down-regulation of DNA repair genes in occupational workers leading to DNA damage and oxidative stress. They have the potential to alter redox balance by generation of Reactive oxygen species (ROS) inside cells, which causes genotoxic effects inside the body. The present study aims to elucidate the relation of *hOGG1* down regulated expression profile with single nucleotide polymorphism in exon 7, at codon 326; locus rs1052133 of *hOGG1* gene located at chromosome 3p25. For this purpose, blood samples (n=20) from brick kiln emissions exposed workers were collected. All disease and health related profiles of workers were noted in a pre-designed questionnaire. DNA extraction from blood samples were carried out using phenol-chloroform method. 1% gel electrophoresis confirmed the extraction of genomic DNA. Primer-3 software https://primer3.ut.ee/ was used to design primers for amplification of *hOGG1* gene to analyse variation at exon 7 of *hOGG1* gene. The general conditions, including annealing temperature, size of amplicon, concentration of salt and length of primers were optimized. The Ensemble website: http://asia.ensemblr.org/Homo_sapiens/Info/Index provided the reference sequence that was essential for building primers. Specificity of the chosen primers was confirmed through BLAT (Blast Like Alignment Tool) on the UCSC genome browser and the In-Silico PCR tool. Polymerase chain reaction was performed on selected DNA samples of five male workers with more exposure time as well as smoking and prevalence of disease were selected for further analysis.

2% gel electrophoresis confirmed the 679 bp PCR product of amplified gene. PCR purification was performed, and samples were sent for commercial DNA sequencing (sanger sequencing) to Macrogen Korea. Analysis of sanger sequencing results revealed C>G transversion at codon 326 in exon 7 of *hOGG1* gene. Furthermore, other sequence variants in this exon were also be identified. In conclusion only 5 samples were analysed. Genomic analysis on large number of samples for *hOGG1* gene exon 7 polymorphism is needed to reveal the role of this sequence variant in increasing risk of DNA damage related health issues in other brick kiln workers.

2.Introduction

According to Climate and Clean Air Coalition Secretariat 2018 report, there are around 20,000 workers association with brick kiln sector in Pakistan which accounts for 1.5 % gross domestic product of the country (David *et al*., 2022). The air pollutants resulting from this sector have serious impacts on public health (Nasir *et al.*, 2021). Brick manufacturing is one of the fastest developing industrial sectors in many countries, such as Pakistan, China and India (Asif *et al.*, 2021). It has been discovered that biomass used in conventional brick kiln sectors is responsible for the emission of greenhouse gases, like carbon monoxide (CO), carbon dioxide (CO_2), methane (CH4), oxides of nitrogen (NO_x), oxides of sulphur (SO_X) and appreciable amounts of hazardous fumes containing suspended solid particulate matter (SPM) rich in carbon substances (Asif *et al.*, 2021; Bashir *et al.*, 2023; Rehman *et al.*, 2021). These pollutants are producing major health issues in Pakistan by disturbing atmospheric air quality (Subhanullah *et al.*, 2022).

2.1 : Brick Kiln Emissions

A brick kiln manufacturing 800,000 bricks on average needs a huge quantity of rubber to start the process of combustion (Bhat *et al.*, 2022). It burns eight tons of coal or 20 barrels of engine oil, emitting various hazardous pollutants such as NOx, CO, and dioxin (Haruna *et al.*, 2023; Maneechot *et al.*, 2020). Air pollutants doubled after increased installation of brick kilns in Peshawar, Pakistan (Saeed *et al.*, 2023). Recent studies have shown that brick kiln industry has increased the concentration of SO_x up to 80 μg/m^3 annually in Pakistan (Hussain *et al.*, 2022). Owing to the combustion of sulfur containing fossil fuels, Sulphur dioxide (SO2) is released into the atmosphere resulting in formation of acid rain which causes serious illnesses, especially respiratory diseases and skin cancer (Shalini *et al.*, 2023). Combustion process in coal-fired power plants results in formation of NOx compounds (Ren *et al.*, 2020; Xu *et al.*, 2022).

Suspended particulate matter (SPM) is fine solid particles with 100 micrometer diameters dispersed in the atmosphere formed due to the combustion reactions (Karuppasamy *et al.*, 2022). It has been documented that large number of heavy metals are released during burning process of oil in brick kilns releases (Haque *et al.*, 2022). A study reported the increased concentration of heavy metals in brick kiln workers (David *et al.,* 2020). Crystalline silicon dioxide (SiO2),

containing crystalline silica (CS) have health devastating impacts on brick kiln workers (Dabić *et al.*, 2020). When organic material is burned it produces polycyclic aromatic hydrocarbons (PAH). The major source of PAH emission is combustion of coal at brick kiln sites (Liu *et al.*, 2022).

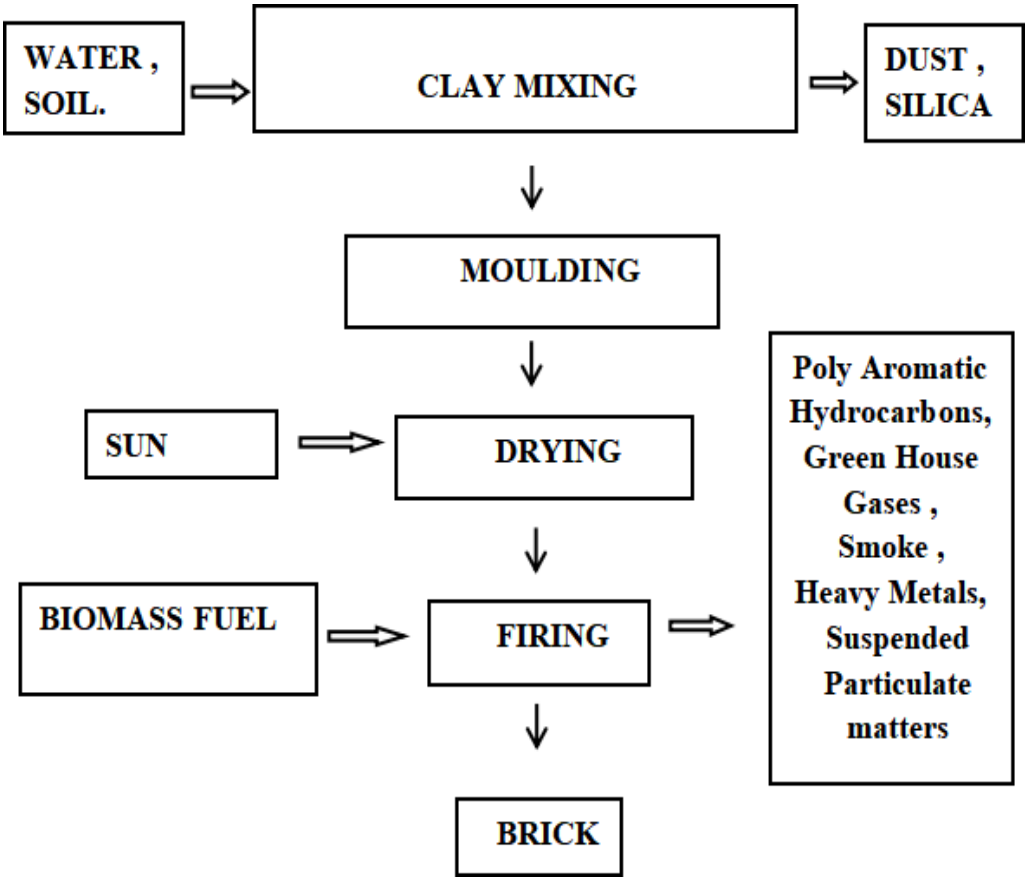

Figure 1: Brick manufacturing process with associated hazardous pollutants

Figure 1: Shows the traditional process of bricks manufacturing and release of different types of pollutants and hazardous chemicals such as greenhouse gases, poly aromatic hydrocarbons, smoke, and heavy metals from burning biofuels. These pollutants, especially heavy metals, are highly toxic and can cause genotoxicity in body cells.

2.2 : Health Impacts of Brick kiln Pollutants

Brick kiln emissions include carcinogenic dioxins, hydrocarbons, SO_x, NO_x, fluoride compounds, CO, and fine dust particles that disrupt the ecosystem and affect negatively on public health (Ahmad *et al.*, 2022; Chowdhury *et al.,* 2022). Issues related to atmospheric air pollution include shortage of oxygen for animal respiration, poor sight, eye discomfort, and rise in upper respiratory tract illnesses (Alves *et al.*, 2023; Singh *et al.*, 2022; Wolkoff *et al.*, 2021). Sulphur dioxide, a major air pollutant, causes lung cancer, cardiovascular disease, and other respiratory illnesses (Khalaf *et al.*, 2022; Khan *et al.*, 2022). Gases like CO and CO_2 have negative impacts on cardiovascular, neurological, and respiratory systems (Kumar *et al.*, 2023). Laryngeal cancer, asthma, emphysema, chronic bronchitis, wheezing, and dyspnea are only a few respiratory problems caused by brick kiln emitted gases (Jehangir *et al.*, 2019; Johnston *et al.*, 2020; Rajak *et al.*, 2020). It has also been reported that SPM is associated with child mortality rate and health complications (Anwar *et al.*, 2021; Aslam *et al.*, 2021; Main *et al.*, 2023). Heavy metals emission from this sector has negative impacts on hematological and hormonal profile of workers in Pakistan (David *et al.,* 2022).

Polycyclic aromatic hydrocarbons (PAHs) can cause oxidative stress and inflammation inside body cells, by altering the redox balance and generation of reactive oxygen species (ROS). These increase the risk of a variety of illnesses, especially in individuals with metabolic disorders (Zhang *et al.*, 2020). A few unfavorable outcomes, notably cancer (Stading *et al.*, 2021) cardiovascular disorders (Chen *et al.*, 2021) and type 2 diabetes (T2D) (Cheng *et al.*, 2021) have been associated with exposure to PAHs emission from multiple industrial sectors. Exposure to high concentrations of heavy metals can have negative impacts on health of living organisms due to their hematotoxic, genotoxic and reprotoxic potential (Chen *et al.*, 2023; David *et al.*, 2020; David *et al.*, 2021; OO *et al.*, 2021) For example, exposure to lead (Pb) induced toxicity has detrimental effects on several organs and organ systems, including immunological system, reproductive system, and cardiovascular system (Sarkar *et al.*, 2023).

2.3 : DNA Damage due to Brick Kiln Emissions

Reactive oxygen species (ROS) are byproduct of cellular metabolism, exposing to carcinogenic environment, UV and ionizing radiation, and other environmental factors (Anik *et al.*, 2022). Brick kilns use hazardous raw materials during firing process of bricks, which have an adverse effect on the health of individuals (Parvez *et al.*, 2023). ROS and oxidative DNA damage to cellular systems are two primary outcomes of metal-induced toxicity (Makhdoumi *et al.*, 2020). Heavy metals bind to nuclear proteins and DNA to induce oxidative degradation of biological macromolecules (Engwa *et al.*, 2019). When metals attach to sulfhydryl groups in proteins, it can cause toxicity by inhibiting function or inactivating structure (Ilieva *et al.*; B. Zhang *et al.*, 2022). Burning of oil in brick kilns results in significant quantities of heavy metals which have genotoxic consequences (Kabir *et al.*, 2022). DNA bases are more prone to damage caused by ROS (Fleming *et al.*, 2020).

A study reported a significant elevated DNA damage in lymphocytes of workers exposed to brick kiln emissions and recorded a positive correlation between genotoxicity and time duration of occupational exposure (Khisroon *et al.*, 2018). However, it has been found that there is no association between lymphocyte DNA damage and age; hence, heavy metals emission from brick kilns constitutes the root cause of genotoxicity (Anukriti *et al.*, 2019) Another investigation aimed to identify arsenic-exposed DNA fragmentation and oxidative stress caused by metal exposure in industrial workers. The findings revealed reduction in antioxidant level and an elevation of DNA damage in industrial workers (Raza *et al.*, 2018).

Malignant cells are thought to arise because of genetic material being damaged by crystalline silica through inflammation and ROS. Research found the evidence of DNA damage in workers exposed to crystalline silica, including greater levels of 8-oxoG and DNA adducts with malondialdehyde (Wultsch *et al.*, 2021). It has also been found that polycyclic aromatic hydrocarbon (PAH) also causes DNA damage (Nsonwu-Anyanwu *et al.*, 2022).

2.4 : Transversion of DNA Bases by Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are highly reactive in nature and can cause DNA damage to cells (Anik et al., 2022). DNA bases are more prone to damage caused by ROS because

they bind with functional groups of proteins and DNA and cause structural changes to it (Fleming *et al*., 2020). Owing to lower redox potential guanine is susceptible to oxidation due to high concentration of ROS (Rozelle *et al.*, 2021). Oxidized guanine (8-oxoG), mimics thiamine base and has a potential to mismatch with adenine base during DNA replication, resulting in transversion variation of G:C to T:A base pair (Zhang *et al.,* 2021). Substitution of two-atoms occur in guanine base (an addition of hydrogen atom on nitrogen atom at position 7 (N7) and addition of oxygen atom on the carbon at position 8 (C8) occurs, which makes 8-oxoG to mimic Thiamine and base pair with adenine (Neeley *et al.*, 2006). Numerous studies observed that replication of oxidized DNA bases such as 8-oxoG leads to transversion of GC to AT and GC to TA variations (polymorphism) in oxidative stress exposed cells (Neeley *et al.*, 2006).

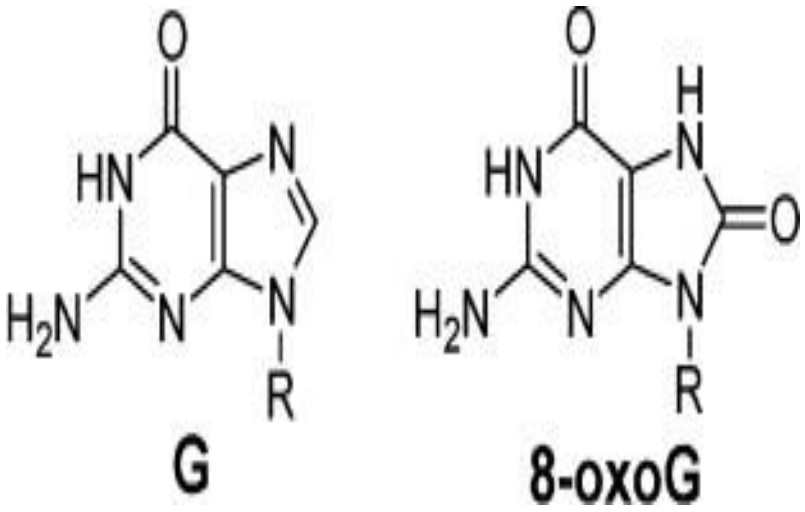

Figure 2: Oxidized Guanine base

Figure 2: Demonstrates the structural differences between guanine and oxidative product of guanine base (8-Oxo- G). The only difference is the presence of oxygen atom on carbon at position 8 and presence of hydrogen at position 7 on nitrogen atom. (Neeley *et al.*, 2006).

This alteration of base sequence creates multiple single nucleotide polymorphisms (SNPs) in genomic sequence of genes which can have beneficial or harmful impacts on normal functioning of proteins.

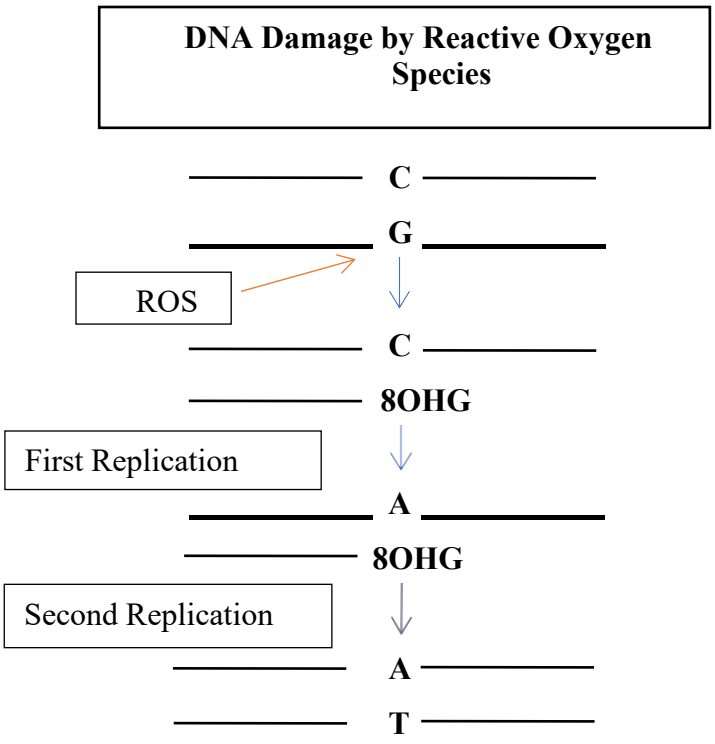

Figure 3: Transversion of GC base pair with AT base pair due to oxidation of guanine base through reactive oxygen species. This transversion leads to various DNA damage consequences including genetic mutation, enzyme repair activities, apoptosis, and cancer (Zhang *et al.,* 2021).

2.5: Human *OGG1* Gene Structure (*hOGG1)* and Function

hOGG1 DNA repair gene, located on chromosome 3p25, encodes enzyme with glycosylase or AP lyase catalytic mechanism responsible for the repair of oxidized DNA bases through BER pathway (Zhao *et al.*, 2022). It has seven exons, six introns and two isoforms of protein results from alternative splicing of single mRNA, i.e., alpha and beta isoforms. The variant *α-hOgg1* encodes peptides of 345 amino acids and *β-hOgg1* encodes 424 amino acids (Abduljaleel, 2019). Transcribed *α-OGG1* has seven exons, while *β-hOGG1* has the first six exons similar to α-form, but due to alternative splicing, the 7^{the} exon is substituted by a new exon

8 (Fig. 4). The *α-hOgg1* protein has a targeted nuclear localization signal (NLS), while *β- hOgg1* has the mitochondrion localization signal (MTS). Thus, *β-hOgg1* is responsible for DNA base repair activity especially 8-oxo-guanine repair reported in mammalian mitochondria cells (Boiteux *et al.*, 2000).

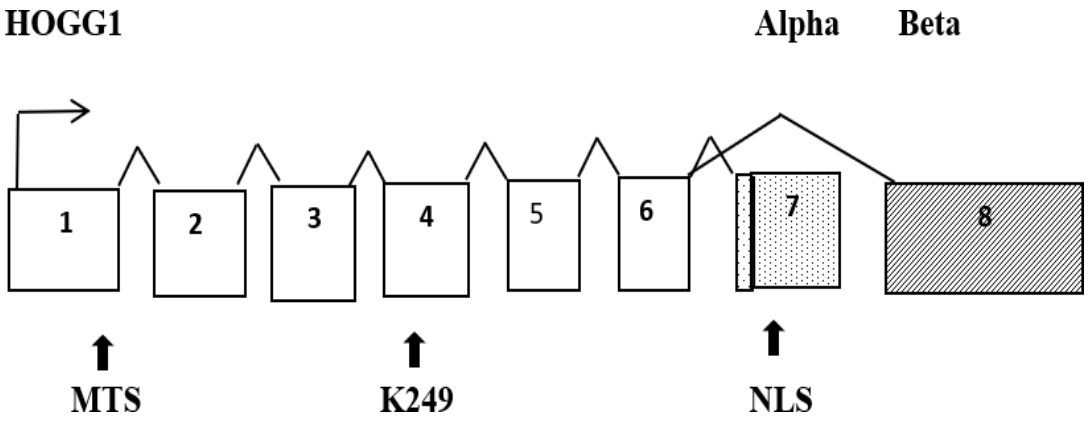

Figure 4: Genomic structure of human *OGG1* Gene.

2.6: *hOGG1* and Base Excision Repair Pathway

Base excision repair pathway (BER) is repair of DNA damage (oxidized) bases, especially 8-hydroxyguanine resulting from alkylation, deamination, and oxidative damage (Sedgwick *et al.*, 2007). Repair of 8-Oxo-G through BER pathway is performed through complex mechanisms of enzymatic actions.

Recognition of 8-hydroxyguanine (8-oxoG) by DNA glycosylase and catalytic cleavage of N-glycosidic bond to remove oxidized guanine and creation of apurinic or apyrimidinic site (AP site) is the first step in BER pathway. DNA AP endonuclease or DNA AP lyase cleaves the DNA backbone and form single stranded DNA nick 5' to the AP site, opposes to 3' to the AP site. As a result, a gap is formed with 5'-phosphate at one end and 3'-hydroxyl on the other end. DNA polymerase β aligns at the AP site and adds appropriate base at the gap site. Finally, a DNA ligase (LIGIII) seals the nick at the end of the repair procedure and restore the stability of DNA helix as shown in (Fig. 5) (Robertson *et al.*, 2009).

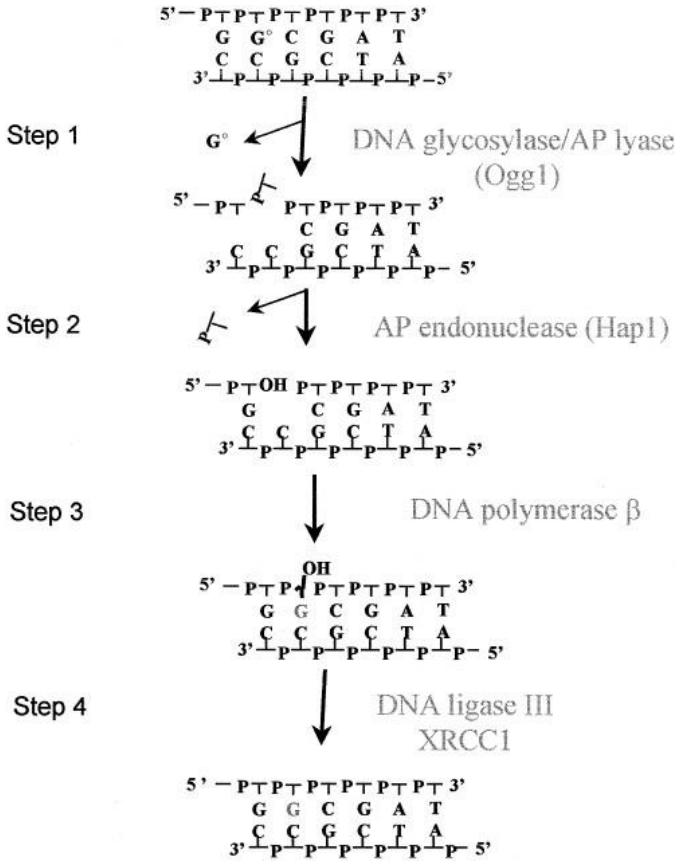

Figure 5: DNA damage base repair via short patch base excision repair pathway (Boiteux et al.,2000)

2.7: Single Nucleotide Polymorphisms in *hOGG1* Gene Exon 7 Role in Diseases

Single nucleotide polymorphism in exon 7 at codon 326; locus rs1052133 was first reported in lung carcinomas (Kohno *et al.*, 1998). It was reported that genetic polymorphism at codon 326 is responsible for impair activity of *hOGG1* enzyme. Occurrence of (SNPs) in the OGG1 gene impairs its DNA repair capacity (Peng *et al.*, 2014). It has been observed that homozygous *OGG1* Ser326Ser genotype exhibited considerably less oxidative DNA damage than the heterozygous variant Ser326Cys genotype in charcoal exposed workers. This research study was conducted on 77 (PAH) exposed charcoal workers in Haryana state of India, which showed frequency of 29 heterozygous variant alleles (Ser/Cys) and 10 homozygous variant *hOGG1* allele (Cys/Cys) with increased DNA damage (Miglani *et al.*, 2021). Research conducted on 40 radiation exposed workers reported occurrence of 57.5% heterozygous variant, 25% homozygous variant and 17.5% wild type alleles. No significant association of *hOGG1* polymorphism with DNA damage was found through comet assay.

It was further reported that age and duration of exposure are strongly correlated with DNA damage, while smoking had no association with DNA damage (Surniyantoro *et al.,* 2022). *h*OGG1 variant genotype (ser/cys) showed significantly increased ($p<0.05$) value of oxidative damage to DNA as compared to those with wild type *hOGG1* (ser/ser) genotype in pesticides exposed agricultural workers (Kaur *et al.*, 2020). High frequency of variant heterozygous (Ser/Cys) genotype as compared to variant homozygous genotype (cys/cys) was observed in radiotherapy exposed workers (Soliman *et al.*, 2020).

Heterogenous SNPS at codon 326 were involved in inactivity of BER pathway that leads to oxidative pathologies (Simonelli *et al., 2013).* Another research study was conducted in Indonesian population for evaluation of correlation of *hOGG1* single nucleotide polymorphisms with thyroid cancer, showing that individuals with heterozygous variant genotype had 7.5 times higher and individuals with homozygous variant had 2.5 times higher risk of thyroid cancer development compared with wild type genotype *hOGG1(*Surniyantoro *et al., 2021)*. Research study reported that the frequency of homozygous wild type allele ser/ser was 55.7%, heterozygous variant allele genotype ser/cys was 36.0% and genotype frequency of homozygous variant allele cys/cys was 8.3%, in Polish population. Results showed that the cys/cys and ser/cys genotypes are strongly related to an elevated risk of laryngeal carcinomas (Pawlowska *et al.*, 2009). *hOGG1* genotype ser/ser had a stronger activity to repair oxidized guanine base than variant cys/cys genotype (Chen *et al.*, 2003).

However, some studies reported contradictory results. Genomic analyses of *hOGG1* gene reported a single nucleotide polymorphism with 75% allelic frequencies of Ser326 and 25% allelic frequency of Cys326 in the Caucasian population and reported that polymorphism had no association with altered *hOGG1* protein activity in human lymphocytes (Janssen *et al.*, 2001). Study conducted in western Maharashtra, India showed 34.5% frequency of heterozygous variant (ser-cys) in cases and 53.0% in controls, the results showed that SNPs in exon 7 have no association with gastrointestinal cancerproliferation (Patil Madhavi *et al.*, 2022).

2.8: Altered Expression Profile of *hOGG1* in Industrial Exposed Workers

Several previous studies already showed that exposure to industrial pollutants, especially heavy metals, leads to genetic variation in *hOGG1* gene. However, reduced expression profile of *hOGG1* gene was also observed in industrial workers, especially brick kiln emissions exposed individuals. Research, involving 80 age-matched women, was done to determine the association of burning biomass with concentration of DNA repair proteins. Results of the study revealed decreased expression of BER proteins with greater percentage of cells expressing DNA damage base 8-oxoG (Mukherjee *et al.*, 2014). Exposure to arsenic affects the redox equilibrium, causes DNA damage, and causes reduction of *hOGG1* expression in individuals exposed to it as compared to controls (Akram *et al.,* 2022). Another study found that employees on construction sites, exposed to lead (Pb), experience more DNA damage, increased levels of oxidative stress anddown regulation of the hOGG1 gene (Akram *et al.,* 2019).

2.9. Rationale of Study

Brick kiln, the fastest growing sector in Pakistan, results in emission of greenhouse gases and pollutants which are posing serious threats to public health (Parvez *et al.*, 2023). Emission of heavy metals from brick kiln industry is causing oxidative stress leading to cytotoxicity and genotoxicity through disturbing the redox balance inside body. Previous studies reported multiple DNA damage related to oxidative stress caused by different environmental factors including brick kiln emissions (Raza *et al.,2018;* Khisroon *et al.,2018). hOGG1* gene located on chromosome 3p25 is involved in repair of DNA damage by removing oxidized bases (8-Oxo-G). Variations related to inactivation of *hOGG1* DNA glycosylases lead to multiple diseases and cancer proliferation in the body. A study reported that downregulation of expression profile of hOGG1 is related to transversion of C>G at codon 326 of exon 7, which causes substitution of amino acid serine into cysteine. Recent studies showed the downregulation of expression profile of *hOGG1* in arsenic exposed brick kiln and other heavy metal exposed workers (Akram et al., 2022; Akram et al.,2019). The present study aims to elucidate the relation of *hOGG1* downregulated expression profile and multiple disease proliferation with polymorphic inactivation of *hOGG*1 at codon 326 in brick kiln exposed workers. The main objectives are extraction of DNA, amplification of *hOGG1* exon 7 using PCR and exposing variations with sanger sequencing.

2. Materials and Methods

3.1 : Blood Sample Collection

After getting the written consent from volunteer brick kiln exposed workers, 5ml blood was collected from study subjects in labelled EDTA vacutainer tubes. Tubes were gently shaken to mix the blood with appropriately labelled EDTA to prevent blood clotting and later stored at -4ºC in the refrigerator until further analysis.

3.2 : Exclusion Criteria

Workers who do not visit brick kiln regularly were excluded.

3.3 : Inclusion Criteria

Brick kiln exposed workers aged between 30-45 years who voluntarily participated in the study.

3.4 : Extraction of Genomic DNA

Phenol-chloroform method of organic extraction was used to extract the genomic DNA. The chemical concentration and composition used in DNA extraction through phenol-chloroform method.

Table 1: Chemical concentration and composition used for genomic DNA extraction.

Sr No.	Solutions	Concentration and chemical Composition
1.	Solution A	Distilled water= 650ml Sucrose (1M) = 320ml Tris HCl (1M) pH 7.5=10ml $MgCl_2$ (500mM) = 10ml 1%(v/v) Trition-X-100 = (10ml)

2.	Solution B	Distilled water = 586 ml Tris HCl (1Mole) pH 7.5 = 10ml EDTA (500mM) pH8.0=4ml NaCl (1M) = 400ml
3.	Solution C	Phenol (pH 8.0)
4.	Solution D	Chloroform: Isoamyl alcohol (24:1)
5.	TE Buffer	10mM Tris HCL (PH=8.0), 0.1 mM EDTA
6.	SDS	20%
7.	Proteinase Kinase	10mg/ml
8.	Ethanol	70%
9.	Chloroform: Isoamyl Alcohol	24:1
10.	Isopropanol (chilled)	100%
11.	Ethanol	70%

The 3-days genomic DNA extraction protocol is as followed.

Day 1.

- EDTA tubes filled with blood samples were kept out of refrigerator for 15 minutes before starting the extraction of genomic DNA.
- For each sample, an empty Eppendorf tube was labeled with the name and placed in wreck in front of the sample to avoid confusion.
- 750 µl blood from each sample was transferred to labelled Eppendorf tube.
- 750 µl of sol A was added in blood and tubes are left for 25 minutes at roomtemperature.
- Centrifugation was done at 13000 rpm for 15 minutes.
- Half of the upper layer after centrifugation was discarded.
- Sol A (400 µl) was again added, and centrifugation was repeated for 13000 rpm for 15 minutes.
- Each time, before centrifugation, the pellet was ensured to be broken by vortex or tapping.
- The washing of blood sample with sol A, was done three to four times until pallet appeared clear.
- Once the clear pallet was obtained, the following chemicals were added.
  - ❖ Solution B (400 µl)
  - ❖ Sodium dodecyl sulphate (SDS) (25µl)
  - ❖ Proteinase kinase (PK) (5 µl)
- All tubes were vortexed and incubated overnight at 37 °C.
- Left the tubes for incubation overnight.

Day 2.

- Overnight left incubated tubes were taken out and placed at room temperature for five minutes.
- Solution D and C+D were made fresh on the same day (day 2) before use.
- 500 µl of Sol (C+D) was added in each Eppendorf tube.
- After adding the solution, centrifugation was performed at 13000 rpm for 15 minutes.
- New Eppendorf tubes were labelled to transfer the upper layer from centrifuged tubes neatly.

- 500 µl of Solution D was added in newly labelled Eppendorf tubes filled with clear layer.
- The tubes were centrifuged at 13000 rpm for 15 minutes.
- Again, new Eppendorf tubes were labelled to transfer newly picked layer.
- The following chemicals were added in each tube.
  - ❖ Isopropyl alcohol (500 µl)
  - ❖ Sodium acetyl (60 µl)
- After adding the above-mentioned chemicals, centrifugation was performed at 13000 rpm for 15 minutes.
- The whole content from all tubes were discarded gently and carefully.
- 70% ethanol (200 µl) was added, and again centrifugation was performed.
- All tubes were left for drying until no bubbles were left seen (this took a 2-3 hours)
- 200 µl TE buffer was added in dried tubes and left forovernight incubation at 37 °C.

Day 3.

- Heat shock was given to extracted DNA in water bath at 70°C for 1 hour. It inactivated thenucleases and prevented DNA denaturation.
- After the heat shock, tubes were placed at room temperature for 5 minutes.
- To mix the genomic DNA and buffer properly, short spin was given to all the tube by centrifuging at 3000 rpm for 2 minutes.
- DNA samples were then stored at -20°C in a properly labelled cryobox.

3.5. Agarose Gel Electrophoresis (1%)

Gel electrophoresis was done after DNA extraction for its confirmation. The protocol is given below.

- To prepare 1% agarose gel, 0.5 gm of agarose powder was added in 50ml of 1X TBE (Tris-Boric Acid EDTA) buffer in a conical flask.
- 1X TBE buffer is prepared from 10X TBE buffer by adding 900 ml distilled water in 100ml of 10X TBE in a 1000ml bottle. While the 10X TBE buffer is made by adding 0.5M EDTA (40ml), 108 gm Tris, 54gm boric acid and distilled water, raising final volume to 1000ml and adjusting pH at 8.
- To make a clear solution, conical flask (covered with aluminum foil) was kept in microwave oven for 2-3 minutes.
- The flask was kept at room temperature for 3 minutes to cool down.
- 3µl of ethidium bromide was added in the flask. This is an intercalating agent used for identification of DNA under UV light (it is overseen carefully, as the chemical is carcinogenic).
- The casting tray and combs were set in gel mold.
- Clear solution freshly prepared was poured in casting tray gently, so that no bubbles form in the tray.
- The poured solution was placed at room temperature for 30 minutes to solidify (polymerization).
- Before placing gel in gel tank (Clever Scientific Limited, CS-3000V), it was filled by running buffer (1XTBE buffer).
- After solidification of gel, combs were gently removed, and gel was carefully placed in gel tank.
- Before loading each sample in well, 3µl of extracted DNA was mixed with 3µl of 6X bromophenol blue (loading dye) dotted on a parafilm.
- Gel electrophoresis apparatus was closed and ran at 120 volts for 25 minutes.
- After completion of running, the gel was carefully visualized under ultraviolet light (UV) by using Gel Documentation System.

Table 2: Composition of agarose gel and other required chemicals

Sr. No	Solutions	Composition
1.	1% Agarose Gel (50ml)	1X TBE (50ml) Agarose (0.5g) Ethidium bromide(2µl)
2.	2% Agarose Gel (50ml)	10X TBE (5ml) Agarose (1.0g) Ethidium bromide(5µl) Distilled water (45ml)
3.	10X TBE	Boric Acid (0.025M,27.5) EDTA (3.6 g, pH 8.3) Tris Base (0.89M, 54g) Deionized water (500 ml)
4.	Gel Running Buffer (1X TBE)	10X TBE (1 part; 10ml) Distilled water (9 part; 90ml)
5.	Ethidium Bromide (50 ml)	Autoclaved filter water (50ml) Ethidium Bromide (0.5g)
6.	Bromophenol Loading Dye (25 ml)	Autoclaved water(25ml) Bromophenol blue (0.087 %) Sucrose (10g)

3.6: FASTA Sequence of Selected Exon

CTTTTTCCGGAGCCTGTGGGGACCTTATGCTGGCTGGGCCCAAGCGGTGAGTGTACC
TAGGTGTCCTCCCTAGGTTTCCTCTCCTCCAGCCCAGACCCAGTGGACTCTTCCACCA
CCGCCCCAGGTGGCCCTAAAGGACTCTCCAGCCACCCCTGTCCCAACCCCAGTGGAT
TCTCATTGCCTTCGGCCCTGTTCCCCAAGGACTCTTCCACCTCCCAACACTGTCACTA
GTCTCACCAGCCCTGACCCCAGTGTACCCTCCTCCCCACACAGACTCCACCCTCCTA
CAGGTGCTGTTCAGTGCCGACCTGCGCCAATCCCGCCATGCTCAGGAGCCACCAGCA
AAGCGCAGAAAGGGTTCCAAAGGGCCGGAAGGCTAGATGGGGCACCCTGGACAAA
GAAATTCCCCAAGCACCTTCCCCTCCATTCCCCACTTCTCTCTCCCCATCCCCACCCA
GTCTCATGTTGGGGAGGGGCCTCCCTGTGACTACCTCAAAGGCCAGGCACCCCCAA
ATCAAGCAGTCAAGAAGGAACAATAAAATAGAAACATTTGTATGGAAAATGCAGTG
AGGAGTGGTAGGGAAGCAGGTGAGGAGGGGACAGGGCAGAGAAACTCCCGGTTCA
GGGAGGGAAGGGGAGCAGGCTGCCCCCAAGCCCTCCCACGCAGAGGATCATGACCC
GAGGTCCAGGGCCCTAGAGCTGGTGGGGCAGTGTGGGGGACAGTTCTGTGCCCGGC
TCCACGCAGCAGTCTCGACAGCAACAGCCAGCTGCCGGCCCTGCATGGGAAGACAG
AACAGAGGTGGCCGCAGGGGCAGGCCCTCCACTCCAGCCCGGGTGCACCTTTGTGG
ACCACCCATGCCCTTCTGCAGAGCCCGCTCCTCACCC

3.7: Primer Designing

Primer-3 software https://primer3.ut.ee/ was used to design primers for amplification of the hOGG1 gene. The general conditions, including annealing temperature, size of amplicon, concentration of salt and length of primer were optimized. The Ensemble website: http://asia.ensemblr.org/Homo_sapiens/Info/Index provided the reference sequence used for building primers. Specificity of the primers was confirmed by using BLAT (Blast Like Alignment Tool) on the UCSC genome browser and the In-Silico PCR tool. Table 3 provides information on the mutation locus, primer sequence, melting temperature (Tm), and size of the product for primers used for hOGG1 gene.

Table 3: Primers for selected mutation in hOGG1 Gene

Gene	Exon	Locus	Primers	Sequence (5'….>3')	Primer length (bp)	Product size (bp)
hOGG1	7	3p25	Forward primer	GACTCTTCCACCTCCCAACA	20	679
hOGG1	7	3p25	Reverse primer	GGCCACCTCTGTTCTGTCTT	20	679

3.8: Primer Dilution

The ordered primers had a commercial concentration of 100 Pico moles/µl, which was diluted to a final concentration of 10 Pico moles/µl.

3.9: Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was done for an amplification of selected exon in study subjects. The procedure was performed in PCR tubes (Axygen USA) having capacity of 200µl. The chemicals of (Thermo Scientific PCR Kit) and the volume used in reaction mixture are given in table 4. It highlights the chemical concentration and volume used for PCR.

Table 4: Chemical concentration and volume used in reaction mixture.

Sr. No	Chemicals	Concentration	Volume
1.	Taq buffer [$(NH4)_2So_4$]	10X	2.5µl
2.	dNTPs	2.5mM	2.5µl
3.	$MgCl_2$	2.5mM	2 µl
4.	Forward Primer	10pmol/ µl	0.5µl
5.	Reverse Primer	10pmol/ µl	0.5µl
6.	DNA	>100ng/ µl	2 µl
7.	Taq Polymerase	5 U/ µl	0.5µl
8.	PCR Water		14.5µl
9.	Total Volume		25 µl

Before placing the PCR tubes in thermos cycle (Bio-Rad T100) for reaction to occur, all tubes were subjected to short spin in centrifuge for 1 minute at 3000 rpm. The thermal conditions set for PCR reaction are given in table

Table 5: Themo cycler profile for Touch Down PCR at 65°C and 55°C

Sr. No	Step	Temperature	Time	Cycle
1.	Initial Denaturation	96°C	5 min	1X
2.	Denaturation	95°C	45 sec	40X
3.	Annealing	55°C	45 sec	40X
4.	Extension	72°C	60 sec	40X
5.	Final Extension	72°C	10 min	1X
6.	Hold	25°C	∞	

3.11: PCR product confirmation

For the confirmation of PCR product, 2% Agarose gel was made by dissolving 1g of agarose in 50ml of 1X TBE buffer and 2µl of EtBr. Gel Electrophoresis was done by loading 2µl of PCR product with 2µl of loading dye; run at 120 V for 25 minutes. Then gel is carefully visualized under ultra-violet (UV) light by using Gel documentation System (Cleaver scientific limited).

3.10 : Purification of PCR Product

A purification kit (Thermo-Scientific) was used to purify the PCR amplified product. The protocol is given below.

- Binding buffer of an equal volume was added to the PCR products.
- The mixture of samples was left at room temperature for 1 minute (after tapping the tubes).
- For proper mixing, tubes were short spin at 13000 rpm for 2 minutes.
- Samples were transferred to pre-labeled spin column tubes with attached collection tubes.
- Centrifugation was performed at 13000 rpm for 1 minute.
- In each sample, 350µl washing buffer was added.
- Tubes were short spin in centrifuge machine at 13000 rpm for 1 minute.
- All samples were kept at room temperature for 2 minutes.
- In each sample, an elution buffer was added (that was kept in an incubator at 70°C before use).
- The spin column tubes were placed in Eppendorf tubes that were pre-labelled.
- The samples were kept at room temperature for 2 minutes.
- Tubes were short spin in centrifuge machine at 13000 rpm for 1 minute.
- To check the purity of DNA, samples were run on 2% Gel (agarose).

3.11 : Sequencing

All the samples were sent for commercial Sanger's sequencing (Macrogen, South Korea). The purified PCR product 8 µl was mixed with 4µl of forward primer, properly sealed before sending for sequencing. The sequence reactions were conducted using big dye terminator chemistry. Capillary electrophoresis method was used to separate the labelled DNA fragments, and a spectrum analyzer was used to confirm their detection. Spectrum analyzer was used for the later detection. For documentation, each nucleotide base (A, G, T and C) was labelled with a specific dye. For visualization of Sanger sequencing results, Sequencher 5.6.4 and Chromas and Chromas 2.6.6 was employed.

3.12 : Genetic Variation Analysis

Ensemble genome browser http://www.ensembl.org/index.html was used to get reference sequence. The sequence to be analyzed were compared against the reference sequence using Sequencher 5.6.4. The detected mutation was confirmed through Mutation Tester. For further confirmations, other computational tools like, PROVEAN https://biotools/provean , and I-Mutant https://gpcr2.biocomp.unibo.it/cgi/predictorsI-Mutant3.0/1-Mutant3.0cgi were used.

4: Results

Blood samples of N=20 male workers exposed to brick kiln emission were selected based on occupational exposure duration and aging between 30-45 years. Out of these N=6 were bakers exposed to smoke, radiation, pollutants, and biofuels and N=14 were molders exposed to clay, silica, and dust particles. Workers working on brick kiln had multiple disease symptoms related to stomach, kidney, joints, and respiratory systems. The analysis of the data showed that individuals who were involved in baking bricks have the highest number of anomalies in comparison to molders as shown in table 6.

Table 6: Demographic characteristics of workers exposed to brick kiln emissions.

Sample ID	Age	BMI	Work Type	Work per hours.	Work Exp. (years)	Exposure type	Health History	Medication Status	Smoking Status
BK-01	43	24.7	Baker	8	27 years	Smoke, fuel, radiation	Bone pain, kidney diseases, respiratory diseases.	None	27 years
BK-02	34	18.7	Molder	6	20 years	Clay, dust particles	None	None	5 years
BK-03	35	20.3	Molder	12	20 years	Clay, dust particles	Bone pain, respiratory diseases	None	20 years
BK-04	39	24.5	Molder	10	2 years	Clay, dust particles	None	None	15 years
BK-05	47	23.5	Molder	7	7 years	Clay, dust particles	Respiratory diseases, stomach	None	22 years

							diseases		
BK-06	31	17.7	Molder	12	14 years	Clay, dust particles	None	None	None
BK-07	39	18.5	Molder	10	17 years	Clay, dust particles	None	None	None
BK-08	42	22	Molder	8	20 years	Clay, dust particles	Respiratory diseases	None	20years
BK-09	40	23	Baker	12	5 years	Smoke, fuel, radiation	Respiratory illnesses.	None	None
BK-10	38	19.8	Molder	12	7 years	Clay, dust particles	None	None	10 years
BK-11	31	18.4	Molder	8	15 years	Clay, dust particles	stomach diseases liver disease, bone pain	None	5 years
BK-12	32	19.1	Molder	6	13 years	Clay, dust particles	None	None	12 years
BK-13	45	19.3	Molder	8	20 years	Clay, dust particles	Kidney diseases	None	8 years
BK-14	33	30.4	Baker	8	20 years	Smoke, fuel, radiation	Respiratory diseases,	None	15 years
BK-15	37	23.5	Baker	10	17	Smoke,	stomach	None	13 years

					years	fuel, radiation	diseases, respiratory diseases,		
BK-16	40	23.5	Molder	12	23 years	Clay, dust particles	Stomach disease (ulcer)	None	20 years
BK-17	45	18.8	Baker	10	20 years	Smoke, fuel, radiation	Respiratory diseases,	None	2 years
BK-18	30	20.5	Molder	10	10 years	Clay, dust particles	Kidney diseases, Bone pain.	None	1 year
BK-19	35	20.6	Molder	12	15 years	Clay, dust particles	Respiratory diseases,	None	None
BK-20	45	30.1	Baker	12	25 years	Smoke, fuel, radiation	Respiratory diseases, stomach diseases	None	15 years

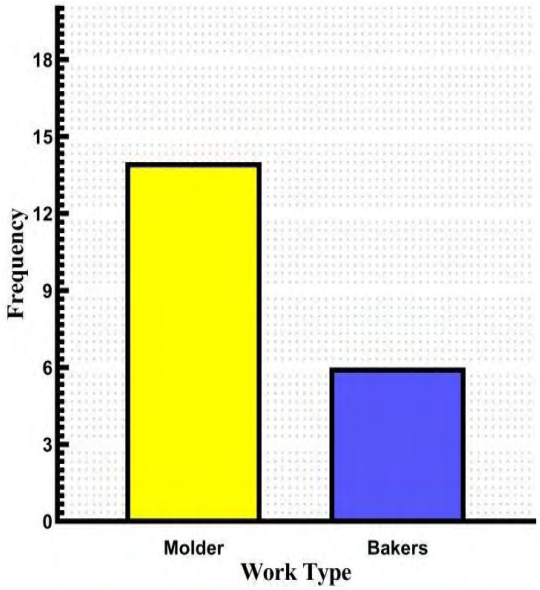

Figure 6: Work type frequency

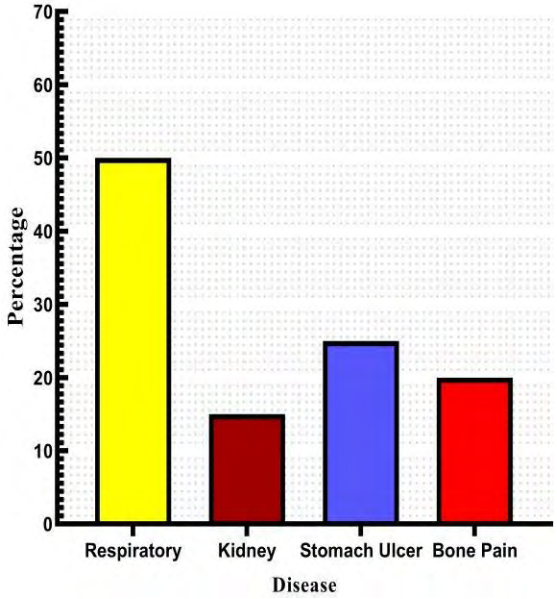

Figure 7: Proportion of disease type in heavy metals exposed brick kiln workers.

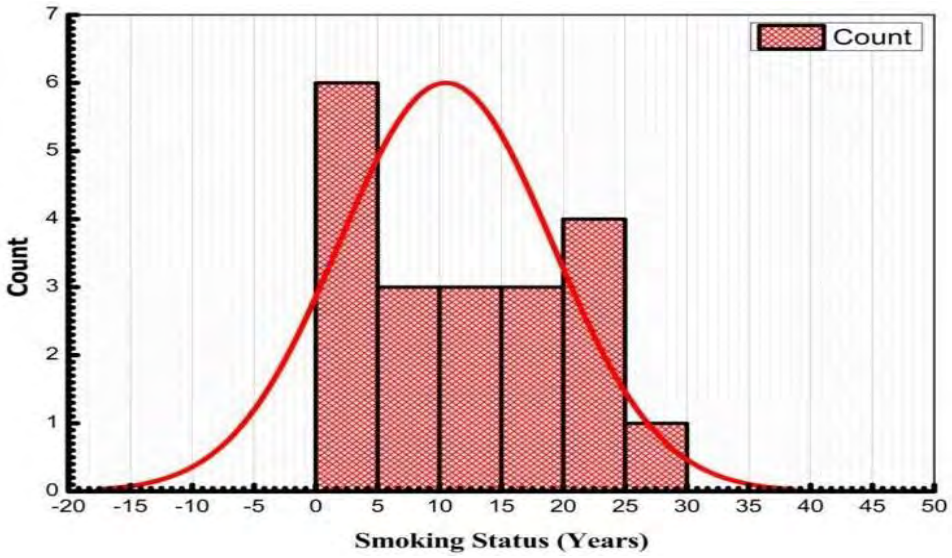

Figure 8: Smoking status among adult male brick kiln workers.

4.1 : DNA Isolation

DNA extraction of twenty samples was done by phenol chloroform method. Confirmation of DNA extraction was done by running extracted product on 1% agarose gel and was checked on gel dock machine for further confirmation as shown in figure 3.1.

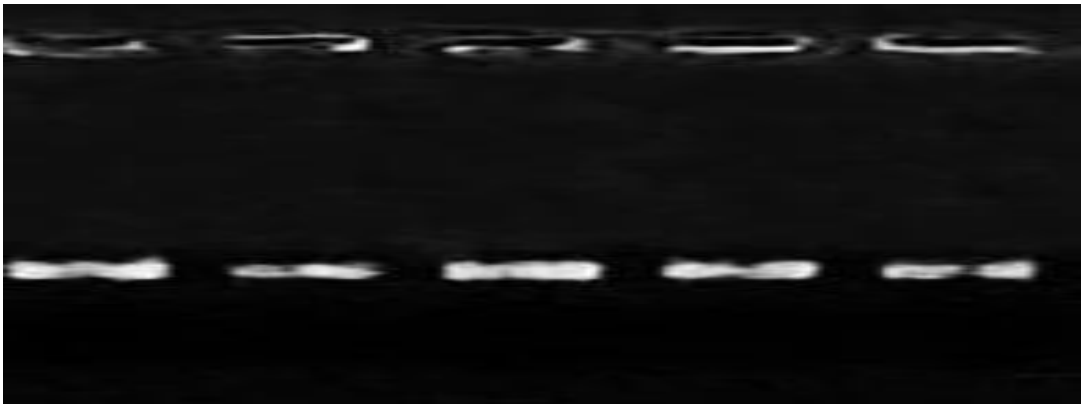

Figure 9: DNA isolated from blood samples of brick kiln workers.

4.2 : Primer optimization

For optimization of primer gradient PCR was used. The annealing temperature 62 was found optimum for primer pair designed for analysis of variations at codon 326 in exon 7 of *hOGG1* responsible for DNA damage repair through BER pathway as shown in figure 9.

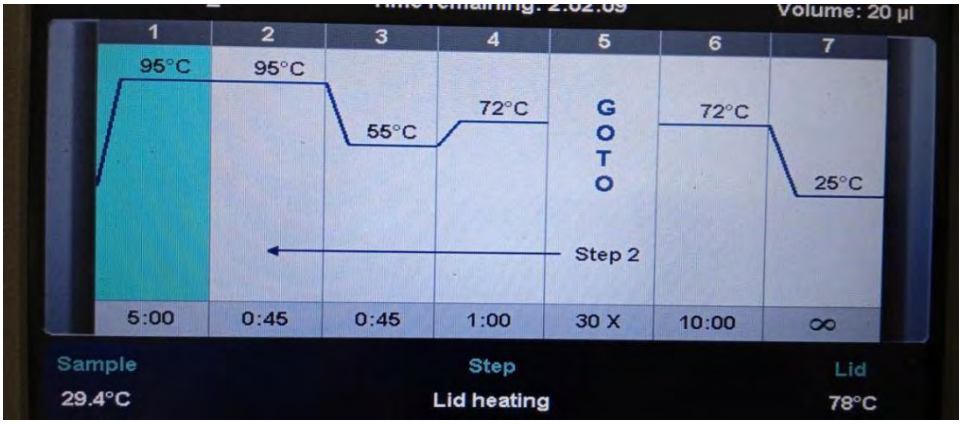

Figure 10: Conditions for PCR cycles

4.3: Polymerase Chain Reaction

Polymerase chain reaction was performed to amplify the DNA samples of five male workers who were bakers and involved in firing of bricks in brick kilns. These five samples were selected based on their exposure duration which was between 15-20 years. The PCR products for analysis of variations at codon 326 in exon 7 of *hOGG1* gene was 679 bp and were obtained by using gradient PCR. The confirmation of amplicon was done by running 2% agarose gel electrophoresis and was observed in gel documentation system under UV light (Cleaver Scientific Limited) as shown in figure 10.

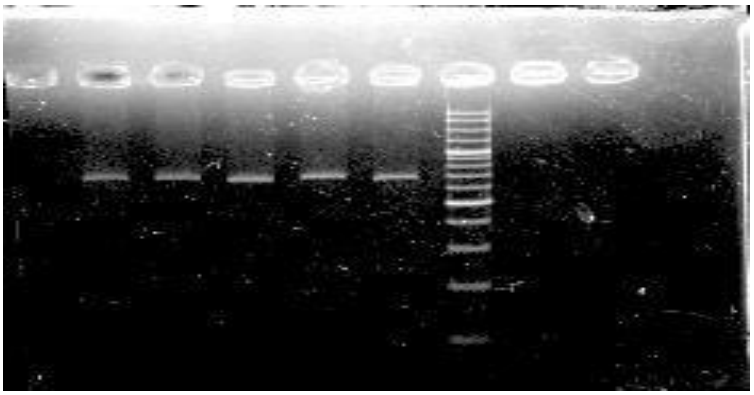

Figure 11: Confirmation purified PCR product of 679 base pair.

5: Discussion

hOGG1 has a crucial role in recognition and excision of 8-oxoG (oxidized base) through base excision repair (BER) pathway (Boiteux et al., 2000). It maintains cellular homeostasis under oxidative stress and avoids the risks of DNA damage. Studies reported that altered expression of *hOGG1* has a negative effect on recycling of oxidized bases (8-oxoG), which leads to DNA damage. This reduction in expression profile of *hOGG1* increases the vulnerability of heavy metals exposed industrial workers including brick kiln industry workers to carcinogenicity (Akram *et al*., 2022; Akram *et al.,* 2020). It is known that multiple external factors are involved in development of cancers including radiation, ultraviolet rays, free radicals, infections, cigarettes, and environmental pollutants (like heavy metals, polycyclic aromatic hydrocarbons, dioxins, and greenhouse gases). Studies confirmed that a reduced or impair activity of DNA glycosylases induces oxidative stress, and multiple diseases including respiratory illnesses, gastric cancer, and prostate cancer etc. (Abduljaleel *et al.*, 2019; Soliman *et al*., 2020; Simonelli *et al*.,2013; Gong *et al.*, 2022). Hence polymorphism in *hOGG1* gene due to emissions from brick kiln industry might increase risk for development of oxidative pathologies.

The mechanism behind the decreased level of expression of *OGG1* is alteration of redox balance due to heavy metal toxicity because they have a potential to bind and oxidize DNA bases. Transversion of cytosine into guanine (C > G) at codon 326 of exon 7 makes *hOGG1* less resistant to oxidative pathologies because change in codon from UCU to UGU makes amino acid cysteine instead of serine in *hOGG1* protein. The presence of sulfur atom in place of oxygen atom in cysteine is the only difference between cysteine and serine amino acid. Cysteine easily oxidized under cellular oxidative stress due to formation of disulfide bridges that alter the configuration of hOGG1 protein and lowers the activity of *hOGG1* S326C variant protein (Simonelli et al., 2013). Another extensive study observed protein stability changes because of substitution of cytosine to guanine in *hOGG1* and its impact on protein structure and stability. It was observed that the Ser326Cys polymorphism results in excessive folding in the variant structure, which causes protein folding particularly in the region of the variation and disrupts protein structure stability (Abduljaleel, 2019).

Multiple studies confirmed that genetic polymorphism of *hOGG1* is linked with decrease enzymatic activity, progression of cancers and oxidative pathologies. Occurrence of

multiple diseases in brick kiln workers exposed to greenhouse gases, polycyclic aromatic hydrocarbons, particulate matters and hazardous chemicals from rubber and heavy metal toxicity might be due to genetic variations in exon 7 at codon 326 of *hOGG1* gene, as study reported that there is a strong correlation of oxidative damage to DNA and *hOGG1* polymorphism with exposure to polycyclic aromatic hydrocarbons. PAHs disturbs the balance between pro-oxidant and antioxidant homeostasis, which causes oxidative damage to cellular and genomic entities (Miglani *et al.,* 2021). Heavy metal exposure to industrial workers leads to decrease antioxidant enzymes levels and increases DNA damage along with downregulation of DNA repair enzyme genes. Research study conducted on biomass fuel exposed workers showed that there is a strong association between PM10 and PM2.5 levels with lower antioxidant enzyme level (SOD) and increased production of ROS (Mukherjee *et al.,*2014). These studies confirmed that brick kiln emission and other industrial emissions have detrimental and negative impact over workers' health.

Therefore, in the present study, brick kiln workers were enrolled for genetic susceptibility analysis. The demographic characteristics and disease symptoms were noted. It was checked that among twenty exposed workers sixteen were suffering from illnesses like respiratory (50%), gastric (25%), kidney diseases (15%) along with bone pain (20%). However, none of these workers were having any type of cancerous disease. Duration of experience, age and other demographic factors also have a role in the development of cancer. As study reported that there is a strong relation of frequency of *hOGG1* gene polymorphism and DNA damage with age and duration of time to industrial emissions exposed workers (Surniyantoro *et al.*, 2022). Another research conducted on *hOGG1* repair activity in lymphocytes cells showed that BER pathway activity of *hOGG1* decreased with age and reported that activity of Hogg1 was significantly reduced in homozygous variant (Cyst/Cyst) genotype (Chen et al., 2003). Even studies also observed association of multiple genes polymorphism was involved in development of cancer. This is a controlling factor for development of cancer by single gene variation. A study reported that genetic polymorphism of *hOGG1* along with other genes polymorphism was associated with development of gall bladder cancer in north Indian population (Srivastava *et al.*, 2010). In present study (n=16) molders and (n=6) bakers were enrolled for genetic analysis. It was found that bakers were suffered from diseases more than molders working at brick kiln because they were more

exposed to biomass fuel, smoke, poly aromatic compounds, suspended particulate matters, heavy metals, greenhouse gases and other hazardous chemicals release from burning of rubber and raw oil. Study reported strong association of smoking with larynx cancer disease. Chemicals from tobacco smoke (Tar) are responsible to oxidize DNA bases and alteration of DNA bases in the *hOGG1* gene. Hence study claimed that smoker's larynx cells are more susceptible to cancer (Pawlowska *et al.,* 2009). Another study also proved that there was a high frequency of *hOGG1* heterozygous and homozygous variants among smoking groups (Sliwinski *et al.*, 2011) Although some studies reported that smoking habit and gender has no role in development of cancer diseases or polymorphism of *hOGG1* gene (Surniyantoro *et al.,* 2022). A study measured no statistically significant differences in level of genotoxicity among smokers and nonsmokers' population (Aka *et al.*, 2004; Janssen *et al.*, 2001). Present study data showed sixteen out of twenty brick kiln workers were extensive cigarette smokers. Figure 8 showed smoking status of workers. Interestingly all the diseases shown in figure () were present in smokers. Thus, at present it cannot be concluded that either smoking is the main factor for occurrence of polymorphism in gene or brick kiln emissions. This parameter should be added in future studies with large sample size.

These Studies are the confirmation that not only environmental factors, but individuals' demographic characteristics are also equally responsible for oxidative damage in *hOGG1* gene and proliferation of multiple oxidative pathologies. The present study amplified the exon 7 of *hOGG1* gene and sent the amplified product for commercial sequencing (sanger's sequencing) to Microgen Korea.

6: References

Abduljaleel, Z. (2019). Structural and Functional Analysis of human lung cancer risk associated hOGG1 variant Ser326Cys in DNA repair gene by molecular dynamics simulation. *Non-coding RNA Research, 4*(3), 109-119.

Ahmad, H. R., Farooqi, Z. U. R., Sabir, M., & Sardar, M. F. (2022). Brick Kilns: Types, Emissions, Environmental Impacts, and their Remedial Measures. In *Biodiversity, Conservation and Sustainability in Asia: Volume 2: Prospects and Challenges in South and Middle Asia* (pp. 945-958): Springer.

Aka, P., Mateuca, R., Buchet, J.-P., Thierens, H., & Kirsch-Volders, M. (2004). Are genetic polymorphisms in OGG1, XRCC1 and XRCC3 genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionising radiations? *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 556*(1-2), 169-181.

Alves, M., Asbell, P., Dogru, M., Giannaccare, G., Grau, A., Gregory, D., . . . Nowinska, A. (2023). TFOS Lifestyle Report: Impact of environmental conditions on the ocular surface. *The Ocular Surface*.

Anik, M. I., Mahmud, N., Masud, A. A., Khan, M. I., Islam, M. N., Uddin, S., & Hossain, M. K. (2022). Role of reactive oxygen species in aging and age-related diseases: a review. *ACS Applied Bio Materials, 5*(9), 4028-4054.

Anukriti, Uniyal, S., Dhasmana, A., Gupta, M., Kesari, K. K., Jamal, Q. M. S., & Lohani, M. (2019). System network biology approaches in exploring of mechanism behind mutagenesis. *Networking of Mutagens in Environmental Toxicology*, 117-136.

Anwar, A., Ullah, I., Younis, M., & Flahault, A. (2021). Impact of air pollution (PM2. 5) on child mortality: Evidence from sixteen Asian countries. *International Journal of Environmental Research and Public Health, 18*(12), 6375.

Asif, M., Saleem, S., Tariq, A., Usman, M., & Haq, R. A. U. (2021). Pollutant emissions from brick kilns and their effects on climate change and agriculture. *ASEAN Journal of Science and Engineering, 1*(2), 135-140.

Aslam, A., Ibrahim, M., Mahmood, A., Mubashir, M., Sipra, H. F. K., Shahid, I., . . . Show, P. L. (2021). Mitigation of particulate matters and integrated approach for carbon

monoxide remediation in an urban environment. *Journal of Environmental Chemical Engineering, 9*(4), 105546.

Bashir, Z., Amjad, M., Raza, S. F., Ahmad, S., Abdollahian, M., & Farooq, M. (2023). Investigating the Impact of Shifting the Brick Kiln Industry from Conventional to Zigzag Technology for a Sustainable Environment. *Sustainability, 15*(10), 8291.

Bhat, M. A., & Gaga, E. O. (2022). Air pollutant emissions in the pristine Kashmir valley from the Brick Kilns. In *Biodiversity, Conservation and Sustainability in Asia: Volume 2: Prospects and Challenges in South and Middle Asia* (pp. 959-979): Springer.

Boiteux, S., & Radicella, J. P. (2000). The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. *Archives of biochemistry and biophysics, 377*(1), 1-8.

Chen, S.-K., Hsieh, W. A., Tsai, M.-H., Chen, C.-C., Hong, A. I., Wei, Y.-H., & Chang, W. P. (2003). Age-associated decrease of oxidative repair enzymes, human 8-oxoguanine DNA glycosylases (hOgg1), in human aging. *Journal of radiation research, 44*(1), 31-35.

Chen, Y.-Y., Kao, T.-W., Wang, C.-C., Chen, Y.-J., Wu, C.-J., Lai, C.-H., & Chen, W.-L. (2021). Polycyclic aromatic hydrocarbon metabolites and mortality risk in an adult population. *Environmental geochemistry and health, 43*, 37-45.

Chen, Z., Wang, F., Zhang, W., Zhou, S., Wen, D., & Mu, R. (2023). Chronic exposure to zearalenone induces intestinal inflammation and oxidative injury in adult Drosophila melanogaster midgut. *Ecotoxicology and Environmental Safety, 251*, 114555.

Cheng, M., Zhou, Y., Wang, B., Mu, G., Ma, J., Zhou, M., . . . Wang, X. (2021). IL-22: A potential mediator of associations between urinary polycyclic aromatic hydrocarbon metabolites with fasting plasma glucose and type 2 diabetes. *Journal of Hazardous Materials, 401*, 123278.

Chowdhury, M. S. S. (2022). *GREEN ECO-BRICK, BRICK KILNS EMISSION AND IT'S ENVIRONMENTAL IMPACT*. International University of Business Agriculture and Technology,

Dabić, P., & Barbir, D. (2020). Implementation of natural and artificial materials in Portland cement. *Hemijska industrija, 74*(3), 147-161.

David, M., Afzal, M., Shoaib, M., Aman, F., Cloete, K. J., Turi, N., & Jahan, S. (2020). Study of occupational exposure to brick kiln emissions on heavy metal burden, biochemical profile, cortisol level and reproductive health risks among female workers at Rawat, Pakistan. *Environmental Science and Pollution Research, 27*, 44073-44088.

David, M., Turi, N., Ain, Q.-u., Rahman, H., & Jahan, S. (2021). Evaluation of environmental effects of heavy metals on biochemical profile and oxidative stress among children at brick kiln sites. *Archives of Environmental & Occupational Health, 76*(7), 441-449.

Engwa, G. A., Ferdinand, P. U., Nwalo, F. N., & Unachukwu, M. N. (2019). Mechanism and health effects of heavy metal toxicity in humans. *Poisoning in the modern world-new tricks for an old dog, 10*, 70-90.

Fleming, A. M., & Burrows, C. J. (2020). On the irrelevancy of hydroxyl radical to DNA damage from oxidative stress and implications for epigenetics. *Chemical Society Reviews, 49*(18), 6524-6528.

Gong, Z., Platek, M. E., Till, C., Goodman, P. J., Tangen, C. M., Platz, E. A., . . . Ambrosone, C. B. (2022). Associations between polymorphisms in genes related to oxidative stress and DNA repair, interactions with serum antioxidants, and prostate cancer risk: Results from the prostate cancer prevention trial. *Frontiers in oncology, 11*, 808715.

Haque, S. E., Shahriar, M. M., Nahar, N., & Haque, M. S. (2022). Impact of brick kiln emissions on soil quality: a case study of Ashulia brick kiln cluster, Bangladesh. *Environmental Challenges, 9*, 100640.

Haruna, A., Tanimu, G., Ibrahim, I., Garba, Z. N., Yahaya, S. M., Musa, S. G., & Merican, Z. M. A. (2023). Mitigating oil and gas pollutants for a sustainable environment–Critical review and prospects. *Journal of Cleaner Production*, 137863.

Ilieva, I., & Sainova, I. Mechanisms of Action of Heavy Metals, Related with Abnormal Protein and Enzyme Activity in Male Infertility Aspect. *Acta Morphologica et Anthropologica, 30*, 1-2.

Janssen, K., Schlink, K., Götte, W., Hippler, B., Kaina, B., & Oesch, F. (2001). DNA repair activity of 8-oxoguanine DNA glycosylase 1 (OGG1) in human lymphocytes is not dependent on genetic polymorphism Ser326/Cys326. *Mutation Research/DNA Repair, 486*(3), 207-216.

Jehangir, F., Syed, H., Zia, A., Arshad, A., & Kamran, N. (2019). Frequency and predictors of copd and restrictive lung disease in primary care. In: PJMD.

Johnston, J. D., Hawks, M. E., Johnston, H. B., Johnson, L. A., & Beard, J. D. (2020). Comparison of liquefied petroleum gas cookstoves and wood cooking fires on PM2. 5 trends in brick workers' homes in Nepal. *International Journal of Environmental Research and Public Health, 17*(16), 5681.

Kabir, M. H., Kormoker, T., Shammi, R. S., Tusher, T. R., Islam, M. S., Khan, R., . . . Idris, A. M. (2022). A comprehensive assessment of heavy metal contamination in road dusts along a hectic national highway of Bangladesh: Spatial distribution, sources of contamination, ecological and human health risks. *Toxin Reviews, 41*(3), 860-879.

Karuppasamy, M. B., Natesan, U., Karuppannan, S., Chandrasekaran, L. N., Hussain, S., Almohamad, H., . . . Abdo, H. G. (2022). Multivariate Urban Air Quality Assessment of Indoor and Outdoor Environments at Chennai Metropolis in South India. *Atmosphere, 13*(10), 1627.

Kaur, K., & Kaur, R. (2020). Impact of single nucleotide polymorphisms in the OGG1 and XRCC1 genes on modulation of DNA damage in pesticide-exposed agricultural workers in Punjab, North-West India. *Biomarkers, 25*(6), 498-505.

Khalaf, E. M., Mohammadi, M. J., Sulistiyani, S., Ramírez-Coronel, A. A., Kiani, F., Jalil, A. T., . . . Derikondi, M. (2022). Effects of sulfur dioxide inhalation on human health: a review. *Reviews on Environmental Health*(0).

Khan, W. A., Shah, S. A., & Khan, A. (2022). In Pakistan, the Transport and Urban Air Pollution Impacts on Human Health and Practical Steps to Avoid Them: A Review. *J. Int. Coop. Dev, 5*, 20.

Khisroon, M., Khan, A., Imran, M., Zaidi, F., Ahmadullah, & Fatima, S. H. (2018). Biomonitoring of DNA damage in individuals exposed to brick kiln pollution from Peshawar, Khyber Pakhtunkhwa, Pakistan. *Archives of Environmental & Occupational Health, 73*(2), 115-120.

Kohno, T., Shinmura, K., Tosaka, M., Tani, M., Kim, S.-R., Sugimura, H., . . . Yokota, J. (1998). Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene, 16*(25), 3219-3225.

Kumar, P., Singh, A., Arora, T., Singh, S., & Singh, R. (2023). Critical review on emerging health effects associated with the indoor air quality and its sustainable management. *Science of The Total Environment, 872*, 162163.

Liu, T., Tian, L., Yang, L., Yan, D., Huang, Q., Lu, G., . . . Huang, Z. (2022). Emissions of BTEXs, NMHC, PAHs, and PCDD/Fs from co-processing of oil-based drilling cuttings in brick kilns. *Journal of Environmental Management, 304*, 114170.

Main, J. I., Ahmad, E., Haider, R., & Khokhar, H. (2023). Seasonal Assessment of particulate matter (PM2. 5) and its contribution towards mortality in Lahore city, Pakistan. *Pakistan Journal of Emerging Science and Technologies (PJEST), 4*(3), 1-13.

Makhdoumi, P., Karimi, H., & Khazaei, M. (2020). Review on metal-based nanoparticles: role of reactive oxygen species in renal toxicity. *Chemical Research in Toxicology, 33*(10), 2503-2514.

Maneechot, P., Thanarak, P., & El Sharkawi, H. M. (2020). Carbonization of Wasted Biomass and Carbon Sequestration. *Recycle Based Organic Agriculture in a City*, 33-68.

Miglani, K., Kumar, S., Yadav, A., Aggarwal, N., & Gupta, R. (2021). OGG1 DNA repair gene polymorphism as a biomarker of oxidative and genotoxic DNA damage. *Iranian Biomedical Journal, 25*(1), 47.

Mukherjee, B., Bindhani, B., Saha, H., & Ray, M. R. (2014). Increased oxidative DNA damage and decreased expression of base excision repair proteins in airway epithelial cells of women who cook with biomass fuels. *Environmental Toxicology and Pharmacology, 38*(2), 341-352.

Nasir, M., Rehman, F. U., Kishwar, S., Bashir, S., & Adil, M. (2021). Air pollution and child health: the impact of brick kiln pollution on children's cognitive abilities and physical health in Pakistan. *Environment, Development and Sustainability, 23*, 13590-13606.

Neeley, W. L., & Essigmann, J. M. (2006). Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chemical Research in Toxicology, 19*(4), 491-505.

Nsonwu-Anyanwu, A. C., Idenyi, A. N., Offor, S. J., Thomas, C. C., Okpotu, F., Edet, C. E., & Usoro, C. A. O. (2022). Association of exposure to polycyclic aromatic hydrocarbons with inflammation, oxidative DNA damage and renal-pulmonary

dysfunctions in barbecue makers in Southern Nigeria. *Reports of Biochemistry & Molecular Biology, 11*(1), 74.

OO, T., AO, A., OP, A., AD, A., & SOA, O. (2021). Effects of Ethanol Extract of Secamone afzelii (Schult) K. Schum (Asclepiadceae) Leaves on Aluminum Chloride-induced Reproductive Toxicity in Male Wistar Rats. *Journal of Krishna Institute of Medical Sciences (JKIMSU), 10*(2).

Parvez, M. A., Rana, I. A., Nawaz, A., & Arshad, H. S. H. (2023). The impact of brick kilns on environment and society: a bibliometric and thematic review. *Environmental Science and Pollution Research, 30*(17), 48628-48653.

Patil Madhavi, N., Datkhile Kailas, D., Bhandurge Parixit, J., Gudur Anand, K., & Gudur Rashmi, A. (2022). hOGG1 and APE1 Gene Polymorphism and Risk of Gastrointestinal Cancer in Rural Population of South-Western Maharashtra. *Research Journal of Biotechnology Vol, 17*, 5.

Pawlowska, E., Janik-Papis, K., Rydzanicz, M., Zuk, K., Kaczmarczyk, D., Olszewski, J., . . . Morawiec-Sztandera, A. (2009). The Cys326 allele of the 8-oxoguanine DNA N-glycosylase 1 gene as a risk factor in smoking-and drinking-associated larynx cancer. *The Tohoku journal of experimental medicine, 219*(4), 269-275.

Peng, Y., Li, Z., Zhang, S., Xiong, Y., Cun, Y., Qian, C., . . . Cheng, Y. (2014). Association of DNA base excision repair genes (OGG1, APE1 and XRCC1) polymorphisms with outcome to platinum-based chemotherapy in advanced nonsmall-cell lung cancer patients. *International journal of cancer, 135*(11), 2687-2696.

Rajak, R., & Chattopadhyay, A. (2020). Short and long term exposure to ambient air pollution and impact on health in India: a systematic review. *International journal of environmental health research, 30*(6), 593-617.

Raza, M., Mahjabeen, I., Fahim, M., Malik, W. A., Khan, A. U., Kayani, M. A., . . . Akram, Z. (2018). Redox balance and DNA fragmentation in arsenic-exposed occupational workers from different industries of Pakistan. *Environmental Science and Pollution Research, 25*, 33381-33390.

Rehman, A., Ma, H., Radulescu, M., Sinisi, C. I., & Yousaf, Z. (2021). Energy crisis in Pakistan and economic progress: Decoupling the impact of coal energy consumption in power and Brick Kilns. *Mathematics, 9*(17), 2083.

Ren, Q., Chi, H., Gao, J., Zhang, C., Su, S., Leong, H., . . . Xiang, J. (2020). Experimental study and mechanism analysis of NO formation during volatile-N model compounds combustion in H2O/CO2 atmosphere. *Fuel, 273*, 117722.

Robertson, A., Klungland, A., Rognes, T., & Leiros, I. (2009). DNA repair in mammalian cells: Base excision repair: the long and short of it. *Cellular and Molecular Life Sciences, 66*, 981-993.

Rozelle, A. L., Cheun, Y., Vilas, C. K., Koag, M.-C., & Lee, S. (2021). DNA interstrand cross-links induced by the major oxidative adenine lesion 7, 8-dihydro-8-oxoadenine. *Nature communications, 12*(1), 1897.

Saeed, T., Abbasi, N. A., Zahid, T., Fatima, N., & Kokhar, M. F. (2023). Toxicological profile and health risk assessment through trace elements exposure in brick kiln workers from Lahore, Pakistan.

Saju, J. A., Rahman, M. M., Debnath, P. K., & Nayan, S. B. IMPACTS OF AIR POLLUTION ON HUMAN HEALTH AND ENVIRONMENT DUE TO BRICK KILNS EMISSION: A REVIEW.

Sarkar, O., Dey, K. K., Islam, S., & Chattopadhyay, A. (2023). Lead and Aquatic Ecosystems, Biomarkers, and Implications for Humankind. In *Biomarkers in Toxicology* (pp. 961-988): Springer.

Sedgwick, B., Bates, P. A., Paik, J., Jacobs, S. C., & Lindahl, T. (2007). Repair of alkylated DNA: recent advances. *DNA repair, 6*(4), 429-442.

Shalini, T. M., Jenifer, J. A., Shiju, B. K., & Gayathri, S. (2023). *Alerting of Acid Rain Using Rain Sensor, pH Sensor and SO2 Sensor on Street Lights.* Paper presented at the 2023 4th International Conference on Electronics and Sustainable Communication Systems (ICESC).

Singh, A., & Singh, K. (2022). An Overview of the Environmental and Health Consequences of Air Pollution. *Iranian (Iranica) Journal of Energy & Environment, 13*(3), 231-237.

Sliwinski, T., Przybylowska, K., Markiewicz, L., Rusin, P., Pietruszewska, W., Zelinska-Blizniewska, H., . . . Majsterek, I. (2011). MUTYH Tyr165Cys, OGG1 Ser326Cys and XPD Lys751Gln polymorphisms and head neck cancer susceptibility: a case control study. *Molecular biology reports, 38*, 1251-1261.

Soliman, A. H., Zaki, N. N., Fathy, H. M., Mohamed, A. A., Ezzat, M. A., & Rayan, A. (2020). Genetic polymorphisms in XRCC1, OGG1, and XRCC3 DNA repair genes and DNA damage in radiotherapy workers. *Environmental Science and Pollution Research, 27*, 43786-43799.

Srivastava, K., Srivastava, A., & Mittal, B. (2010). Polymorphisms in ERCC2, MSH2, and OGG1 DNA repair genes and gallbladder cancer risk in a population of Northern India. *Cancer, 116*(13), 3160-3169.

Stading, R., Gastelum, G., Chu, C., Jiang, W., & Moorthy, B. (2021). *Molecular mechanisms of pulmonary carcinogenesis by polycyclic aromatic hydrocarbons (PAHs): Implications for human lung cancer.* Paper presented at the Seminars in cancer biology.

Subhanullah, M., Ullah, S., Javed, M. F., Ullah, R., Akbar, T. A., Ullah, W., . . . Sajjad, R. U. (2022). Assessment and impacts of air pollution from brick kilns on public health in northern pakistan. *Atmosphere, 13*(8), 1231.

Subramanian, N. Brick Kilns and their Effect on Environment.

Surniyantoro, H., & Hidayati, N. (2021). *Preliminary study in genetic polymorphism of hOGG1 and risk factor for thyroid cancer in Indonesia.* Paper presented at the AIP Conference Proceedings.

Surniyantoro, H. N. E., Yusuf, D., Rahardjo, T., Rahajeng, N., Kisnanto, T., Nurhayati, S., . . . Hande, M. P. (2022). Assessment of hOGG1 Genetic Polymorphism (rs1052133) and DNA Damage in Radiation-Exposed Workers. *Asian Pacific Journal of Cancer Prevention: APJCP, 23*(12), 4005.

Wolkoff, P., Azuma, K., & Carrer, P. (2021). Health, work performance, and risk of infection in office-like environments: The role of indoor temperature, air humidity, and ventilation. *International Journal of Hygiene and Environmental Health, 233*, 113709.

Wultsch, G., Setayesh, T., Kundi, M., Kment, M., Nersesyan, A., Fenech, M., & Knasmüller, S. (2021). Induction of DNA damage as a consequence of occupational exposure to crystalline silica: A review and meta-analysis. *Mutation Research/Reviews in Mutation Research, 787*, 108349.

Xu, J., Zhu, F., Ge, X., Li, H., Zhao, X., Tian, W., . . . Wang, S. (2022). Research progress on volatile organic compounds emissions from coal-fired power plants. *Current Pollution Reports, 8*(3), 303-314.

Zhang, B., Zhang, Q., Zhu, X., Li, D., Duan, X., Jin, J., . . . Liu, Y. (2022). Mechanistic Insight Into Cadmium-and Zinc-Induced Inactivation of the Candida albicans Pif1 Helicase. *Frontiers in Molecular Biosciences, 8*, 778647.

Zhang, H., Han, Y., Qiu, X., Wang, Y., Li, W., Liu, J., . . . Chen, W. (2020). Association of internal exposure to polycyclic aromatic hydrocarbons with inflammation and oxidative stress in prediabetic and healthy individuals. *Chemosphere, 253*, 126748.

Zhang, X., Blumenthal, R. M., & Cheng, X. (2021). A role for N6-methyladenine in DNA damage repair. *Trends in biochemical sciences, 46*(3), 175-183.

Zhao, F., Zhu, J., Shi, L., & Wu, X. (2022). OGG1 in the Kidney: Beyond Base Excision Repair. *Oxidative Medicine and Cellular Longevity, 2022*.