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Expression Analysis of Poly (ADP-Ribose) Polymerase1 Gene in Workers of Salt Mines

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Abstract:- Poly (ADP-Ribose) Polymerase1, PARP1 is an important protein coding gene that involve in DNA repair mechanism. It acts as a first responder that detects DNA damage and then facilitates choice for repair pathways. The current study was design to study the expression variation of PARP1 gene in salt mine workers. In the present study 200 blood samples of exposed salt mine workers along with 200 control groups were used. Expression variety of PARP1 quality was assessed by qPCR. Relative expression of PARP1 was ($p<0.01$) up regulated in exposed groups compared with control groups. Relative expression of PARP1 gene increased significantly in worker group with >30-year age compared to their respective controls ($P<0.01$) and even to worker group with <30-year age ($P<0.05$). The expression of PARP1 gene seen unregulated in mine who work in mine more than 10 years. Study cohort of workers and control group was divided as smokers and non-smokers. Significant ($P<0.05$) up regulation in PARP1 expression was seen in smoker category of mine workers compared with control smokers and non-smokers of mine workers. Mine workers divided into groups based on type of fuel used by workers performing duty as workers using gas or wood. Significant ($P<0.05$) up regulation of PARP1 gene was observed in mine group working with wood compared to group working with gas. The obtained results suggest that salt mine workers are exposed to different genotoxins at their workplaces which affect the genetic integrity and DNA repair capacity.

I. INTRODUCTION

Khewra salt mines, the world's second biggest salt mine, situated 160Km south of Pakistan's capital, Islamabad. The salt mine is 288 meter above the see level and extended about 2438.42 meter inside the mountain. 50% salt is extracted from the mine and 50% left as pillars to keep the mountain (Iftekhar, 1991). The current yearly creation from the mine is 465,000 tones. The salt of khewra mine is clear, white, pink, reddish 99% pure NaCl (Scot and Gray, 2001). Inside the mine there are certain areas that are filled with the brine solution. The water seeps through the walls and roofs and collected in these areas overtime. In brine ponds contain 100 percent pure salt along with enough radon that appears to be transported in the flowing water and then release into the atmosphere of the mine. Since ionizing radiation is present mostly everywhere on earth, such as in rocks, water, soil, and foodstuffs, from primary sources (UNSCEAR, 2000; Baloch *et al.*, 2012). The temperature and humidity of mine in different areas of mine is affected by the explosion of low intensity for the exploration of salt. In the expulsion of salt from these mines, about thousand workers are involved. In these mines, tourists and staff are exposed directly to the inner and outside radiological dangers of radon and gamma beams. The ingestion of salt containing normally happening radionuclides affects the general population (Baloch *et al*, 2012). In mine various radioactive rays, moisture and temperature is very harmful that cause the genome integrity and cause many different diseases. Ionizing radiations are present everywhere and these radiations prompts excitation and ionizations inside the medium which it crosses (Sanders 1988). Assortments of synthetic substance, substantial metals, modern and clinical radiations incite numerous infections (Saeed *et al*., 2014; El-Garawani *et al*., 2017; El Garawani *et al*., 2021; Acharya *et al*., 2010). Workers are directly or indirectly exposed to the radiations, numerous chronic diseases affect the blood cells of workers because of radiations (Mc Hutchison *et al*. 2007; Cadet *et al*. 2010).

PARP is a nuclear protein that binds and catalyzes the formation of frock ADP- ribose polymers from NAD to single and double strands breaks of DNA (Beneke *et al*., 2000). These proteins present in number of eukaryotes. PARP have important role in DNA repair, genome integrity and epigenetic control in eukaryotes (Elsie *et al*., 2010). The PARP has a 17 members family, different in structure composition, sub cellular activities and function (Gibson and Kraus 2012;Vyas *et al*., 2014).Because of their catalytic activity, PARP family members can also be categorize such as mono poly on inert (Vyas *et al*., 2014).PARP1 is one of the best-known member of PARP family and is confirmed to account for at least 85% of PARP cell activity (Banin *et al*., 1998). It is ubiquitous protein and has a distinct biochemical activities make it suitable for both structural and regulatory role in genome (Hassa and Hottiger, 2008; Kim *et al*., 2005; Schreiber *et al*., 2006).PARP1modulate its enzymatic activity and change function of acceptor proteins because of high negative charge and high complexity of ADP ribose polymers (Bai, 2015). PARP1 acting as a docking molecule and through enzymatic modification regulate target protein (Hassa&Hottiger, 2008). It is a nuclear protein which attach post-transnationally to a negatively charged polymer to various target proteins and to itself also. Because of this poly (ADP)ribosylation (PARylation) activity, PARP1 plays its critical role in Single strand break repair, Nucleotide excision repair, Double strand break repair and replication and chromatin structure modulation (Chaudhuri &Nussenzweig, 2017). In the presence of different activators, including DNA damage, the activity of PARP1 is significantly reviving (Amours *et al*., 2005).PARP1 modulate the activity of its substrate to control cellular functions such as DNA damage, transcriptional regulation, and cell death (Schreiber, 2006). PARP1 regulate gene expression at multiple levels such as in chromatin structure, methylation pattern, transcription factors, insulation, and post transcriptional RNA modification (Caiafa

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&Guastafierro, 2009). To interact with other proteins, PARP1 enzymatic activity is regulated by phosphorylation, methylation, acetylation, and ubiquitination (Piao & Fujioka, 2018).

PARP1 is important for maintaining genome integrity and hence involved in number of DNA repair pathways such as base excision repair (BER) and Double strand break (DSB) repair, nucleotide excision repair (NER), (De Vos *et al*., 2012; Beck *et al*., 2014).DNA mismatches repair (MMR), Homologous Recombination (HR) and maintenance of replication fork stability (Liu *et al*., 2011; Hochegger *et al*., 2006; Ronson *et al*., 2018). PARP1 regulate cellular function including DNA repair pathways (Pascal, 2018). During DNA replication the PARP1 recognize Okazaki fragments and promote repairing mechanism (Hanzlikova *et al*., 2018). Emissions of radioactive rays in salt mine will increase the rate of DNA damage and may inhibit the DNA repair mechanism which ultimately ends into carcinogenesis. The main purpose of this study is to examine the articulation analysis of PARP1 gene in salt mine workers and compared to the unexposed controls groups. Many demographic factors like age smoking status, exposure time and working environment greatly enhance the mine induced genotoxic effect.

II. MATERIALS AND METHODS

A. Sampling area and study design

Present study was conducted on workers of Khewra Salt Mines situated in Khewra, north of PindDadan Khan near Jhelum. This study was approved by Ethical review board of COMSATS University Islamabad and collaborating salt mine. Mine workers are occupationally exposed to different chemicals and radiations at their workplaces. The study comprised of two main groups. Exposed mine workers and unexposed control subjects. Total 200 samples were collected from salt mine workers along with age and gender matched control individuals. Data was further sub-grouped based on age, coverage time and smoking category of studied population. Individuals who participated in this study were informed properly and consent forms were obtained. Performa was filled for every person comprising gender, age, exposure time, medical history, addictions, and other habits. After obtaining informed consent, 3ml of fasting venous blood was collected in ethylenediaminetetraacetic acid (EDTA) tube, and the samples were immediately transported to the laboratory in ice cooler box and then stored at -80℃.

B. Expression Analysis of PARP1 gene and RNA extraction

Expressional analysis of PARP1 gene was analyzed using quantitative polymerase chain reaction (qPCR). Expressional variation was evaluated in salt mine workers and compared with their respective control group. For RNA extraction Trizol chloroform method was used. RNA stability and integrity was confirmed by running extracted RNA samples on 1% agarose gel. cDNA synthesis was confirmed by amplification of β-actin using reverse transcription polymerase chain reaction. PCR products were visualized on 2% agarose gel electrophoresis to measure the specificity of reaction. Coding sequences of PARP1 and Beta- actin genes were retrieved from ensemble genome browser, and primers were designed by primer quest tool of IDT (Integrated DNA Technology (Ishrat Mahjaben *et al*., 2012).

C. Statistical analysis:

Statistical analysis was representing with Graph Pad Prism software. After organizing, statistics were analyzed by applying ANOVA (one way) to observe the *PARP1* expression variation between salt mine workers and control subjects and correlation of PARP1 gene with different demographic parameters. Statistical significance value was recognized as $P<0.05$.

III. RESULTS

In this study cohort, 200 salt mine workers with age and gender matched control 200 healthy individual were collected. The demographic details of exposed workers and controls are given in table 1.

Parameters	Control	Mine workers
Total Sample size	200	200
Mean age (years)	36.21	33.89
Age (≤30 year)	78	86
Age (≥30 year)	122	114
Smokers	88	140
Non-smokers	112	60
Total exposure (≤10 year)	---	102
Total exposure (≥10 year)	---	98

Table 1 : Demographic parameters of present study

- Expression of PARP1 gene in exposed salt mine workers

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The study was design to analyse the expression of PARP1 gene in salt mine workers. The expression of PARP1 gene seen upregulated in salt mine workers compared to the control.

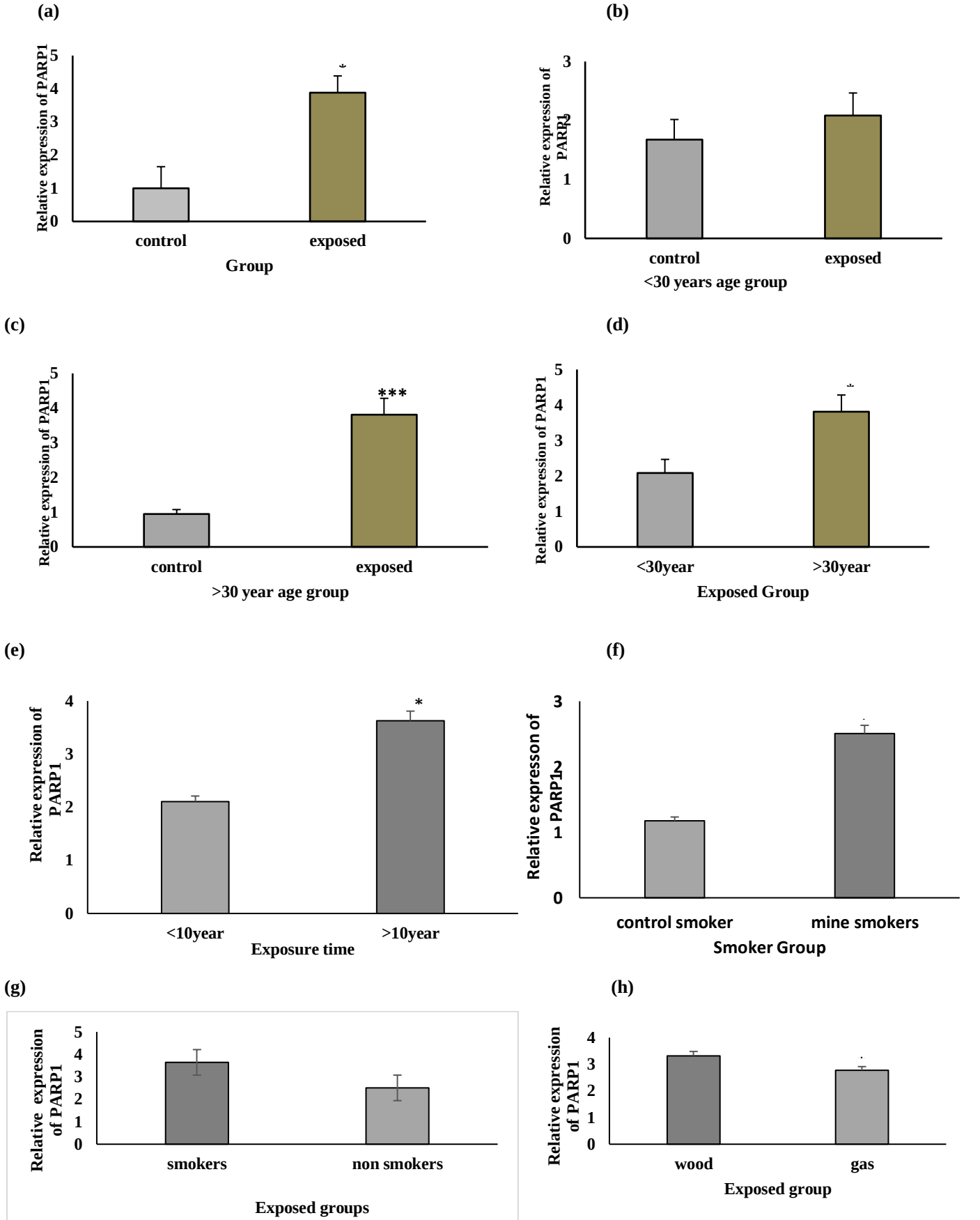

Fig.1 (a) Expression of PARP1 gene was seen in 200 salts mine workers beside with 200 age and sex matched control using quantitative PCR. Relative expression of PARP1 gene was observed extensively (P<0.01) upregulated in salt mine

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workers compared to respective control group. **(b)** Relative expression of PARP1 gene was up regulated in salt mine employees with <30 years of age compared to control subjects with <30 years of age.**(c)**Relative expression of PARP1 gene was observed extensively (P<0.01) up regulated in salt mine workers with >30 years of age compared to control subjects with >30 years of period.**(d)**Relative expression of PARP1 was upregulated extensively (P<0.05) in exposed workers with >30 year of age compared to mine workers with <30 years of age.**(e)**Relative expression of PARP1 was extensively (P<0.01) down regulated in in salt mine labors with ≤10 years of exposed compared to mine labors with ≥10 years contact.**(f)** Relative expression of PARP1 gene was observed extensively (P<0.01) upregulated in smokers of uncovered group compared to smokers of control group.**(g)**Relative expression of PARP1 gene was found extensively (P<0.05) upregulated in smokers of exposed group compared to non-smokers of exposed group.**(h)** Mine worker's population of present study was categorized into two groups based on exposure/working with gas and wood while to perform duty at workplaces. Relative expression of PARP1 gene was observed extensively (p<0.05) upregulated in exposed group working with wood compared to mine workers exposed to gas while working in mines.

IV. DISCUSSION

Khewra salt mine is the world's second largest and Pakistan largest salt mine (Scot and Gray, 2001). Mine environment comprised radiations, high heat, temperature, high salt concentration, different metal, chemicals, and number of other toxins (UNSCEAR, 2000; Baloch *et al.*, 2012; El-Garawani et al. 2017; El Garawani et al. 2021). Gamma radiations deform DNA and chromosomes and destroy internal organs like kidney (Semenova et al. 2020). Thousands of workers have been employed in mines and carry out different duties in different areas of mines. Mine workers, staff, and tourists and to some extent the community living nearby mines is directly or indirectly exposed to different internal and external threats of this toxic environment. Exposure to radiological threats of radon and gamma rays, ingestion of salt containing naturally occurring radionuclides affects the general population (Baloch *et al*, 2012). These radioactive rays, moisture high heat, temperature and salt is very harmful and affect the genome integrity may induce DNA damage and restrict DNA repair ability ultimately leading to carcinogenesis. Maintenance of genomic integrity highly dependent on efficacy of DNA repair systems and inoperative DNA repair system is a risk factor for many types of infection. PARP1 is an important member of the DNA repair mechanism which maintains the stability of the cellular genome. It engaged well in base excision repair pathway or singlestrand break repair. Upon binding to DNA lesions, PARP-1 becomes catalytically active, thus act as a DNA damage sensor (Dantzer*et al.*, 2006). Therefore, it is worth to evaluate the expression variation of PARP1 gene in exposed salt mine workers and its comparison with unexposed individuals. In addition to find the association of PARP1 gene expression with various study demographics likes age, exposure time and smoking status.

Present study discusses the biological role of PARP1 gene in salt mine workers. Relative expression of PARP1 gene was found upregulated in mine workers compared to control subjects. Mine workers are exposed to different toxins at their workplaces on daily and regular basis. Exposure to such toxicants even at very low concentrations but for prolong period of time is enough to induce genotoxic effect and may induce alterations in expression of various genes which may increase the susceptibility of exposed workers to several diseases (Stratford, 2018). Role of poly (ADP-ribose) polymerase1 (PARP1) was highlighted in different pathways of DNA repair. PARP1 is a multifunctional enzyme expressed in eukaryotes (Amours *et al*., 1998) and plays its pivotal role in single strand break repair, nucleotides excision repair, double strand break repair and replication and chromatin structure modulation (Chaudhuri &Nussenzweig, 2017). In the presence of different activators, the activity of PARP1 is significantly reviving. The induction of different kinds of DNA damage result in the recruitment of PARP1 via its DNA binding capacity to damage sites. The condition in which high degree of DNA damage was observed possibly due to severe oxidative stress, it will promote hyper activation of PARP1 which bring about depletion of its substrate NADP and consequently ATP, resulting into necrosis or cell death. These findings support the upregulation of PARP1 activity in exposed mine workers compared to unexposed control.

Present study cohort was divided into different groups based on age, total exposure time and smoking status. Expression of PARP1 gene was upregulated in mine labors with >30 year of age compared to the workers of <30year age. Similar increased expression of PARP1 was noted in laborswith >10-year exposure compared to mine labors with <10-yearexposure.Age and exposure time has been directly associated to one another. Findings of present study suggest the possible involvement of age and work experience in deregulation of PARP1expression. Possibly with age the DNA repair capacity may be affected which act synergistically with prolong occupational exposure to initiate deregulation in PARP1 gene and restrict the genomic stability.

Smoking is an important variable to be considered in biomonitoring studies. Tobacco smoke contains high number of carcinogenic and mutagenic substances (Khisroon et *al*., 2018). Smoking can add to the spread of many human diseases such as lung cancer, cardiovascular disease, and asthma. In present study, smokers of the occupationally uncovered mine workers group reveal increase expressionof PARP1gene contrasted to nonsmokers of their own group. Similarly, theexpression of PARP1 gene was found to be upregulated in smokers of exposed group compared to smokers of the unexposed control group. The exact role of PARP1 in cigarette smoke induced cell injury is still not exactly known. Smoking is known to contribute for increase in ROS generation and utilize polyphenols present within the cell. Tobacco smokes contain many sweet-smelling compounds, unsaturated aldehydes,

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receptive oxygen (ROS) and nitrogen species (RNS) (De Vos *et al*.,2012) which incited oxidative damage to lungs (Cantin*et al*.,2010; See*et al*., 2011). Different parts of tobacco smoke, for example, nicotine and acrolein have additionally been displayed to apply direct genotoxic impacts (Demirhan*at al*., 2011). Mine workers of present study cohort perform different duties at their workplaces. Based on this mine workers were divided into two groups. One group was exposed/working with gas and the other group was working in area exposed to wood combustion. Relative expression of PARP1 gene was observed significantly upregulated in workers engaged with wood combustion compared to workers exposed to gas work.

V. CONCLUSION

Present study demonstrates that mine induced toxicity alter the PARP1 expression in mine workers professionally exposed to various toxicants at their workplace in my environment. In addition, age, smoking and exposure time acts collaborative to make the health of labors at great chance might enhance the degree of DNA damage and restrict the capacity of DNA repair mechanism. Furthermore, present studysuggests the importance of PARP1 in mine induced toxicity and would benefit future research on occupationally exposed workers to manifest their health-related issues.

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