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Determination of levels of C-Reactive Protein (CRP) and Liver function test parameters among occupationally exposed stone quarry workers of Central India: A quest in pursuit of Biomarkers of occupational diseases of stone quarries

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ABSTRACT
Silicosis and other occupational diseases are more prone in stone quarry workers. The prevalence of silicosis varies from 3.5% in ordnance factories to 54.6% in the slate pencil industry. Despite its high prevalence, the unwillingness to use the personal protective equipment and increased quantum of dusts in the environment, pose serious health problems. Biomarker study among Silica exposed workers is limited especially in Indian population and hence the authors were willing to investigate these populations in pursuit of effective biomarker. Total 209 samples were collected from stone quarries of Central India. The workers who were occupationally exposed to Stone quarry dusts were grouped as Experimental (n=101), while rest of the subjects (n=108) were grouped as Control who didn't have history of dust exposure but were residing in that area. Blood samples were collected and serum samples were used for analysis of CRP and liver function test parameters, Sodium fluoride sample was used for random blood sugar analysis. All the liver function test parameters, random blood sugar and CRP levels were found to be higher among exposed population compared to Control. Insignificant positive correlation (r= 0.077, p =0.439 i.e. P value >0.05) was obtained between age and CRP while significant positive correlation was found between years of exposure to stone quarry dust and CRP (r= 0.20, p =0.04 i.e. P value <0.05). No significant correlation was found between liver function test parameters and CRP. Smoking has additive effect on increase in CRP level among exposed workers. Exposure to stone quarry dust alters biochemical profiles causing high level of serum CRP. Determination of serum CRP levels along with liver function test parameters may give evidence about the severity of disease. Male workers are at greater risk of development of occupational diseases especially silicosis as compared to females.
Keywords: *Biomarker, C reactive protein, Liver, SGOT, Silicosis, Stone quarry*

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INTRODUCTION

In India lots of stone quarries are operated to extract stones, marbles, quartz etc by using the mining operations such as drilling, blasting, extraction, tunnelling and so on. As per composition, stones of stone quarry units in India are composed of Silica, Iron, Lead and other trace metal-ores along with impurities. Among these, silica mineral is present in bulk quantity (47%) and as per its deposition, silica is second most abundant mineral in the Earths' crusts (27.7%) [1,2]. According to World health Organization (WHO), workers who got exposed to stone quarry dust, have the chances of development of occupational diseases especially silicosis. It is a non reversible, auto-immune, slowly developing occupational lung disease which affects mainly Miners and Quarry workers [3]. Considering the causes of silicosis, inhalation of respirable crystalline silica dust is the major cause of development of silicosis. Workers in stone crushing units, stone quarries, construction and ceramics are at higher risk of occurrence of silicosis. Nearly ten million workers in India are at risk of developing silicosis, a deadly disease often mistaken for Tuberculosis (TB). Silicosis and TB often occur simultaneously causing an additional menace to the host in the form of Silico-Tuberculosis. It has also been reported that person suffering from silicosis are more prone for HIV infection along with TB [3,4]. According to Sivanmani K., Rajathinakar V., (2013), in India very few epidemiological literature are available in silicosis which reported that the prevalence of silicosis

varies from 3.5% in ordnance factories to 54.6% in the slate pencil industry [5]. Under-reporting of the disease pose a problem to know the true prevalence of silicosis. The incidence rate of silicosis can be drawn from the fact that slate pencil industry in Mandsaur in Madhya Pradesh (India) reported a 59% prevalence of silicosis while Karaoli District of Rajasthan (India) is regarded as Widow Village of West India as most of the men in the area were stone-crushers who expired of silicosis. Also, Andhra Pradesh has one village which is known as Widow's Village [4,6]. Singh MP. [7] reported that higher morbidity levels were observed in ex-quartz factory workers compared to current workers in Gujarat.

C-reactive protein (CRP) as an inflammatory biomarker:

CRP is an acute-phase protein synthesized by hepatocytes in response to circulating Interleukin (IL)-6, IL-1, and Tumour Necrosis Factor (TNF α) having molecular weight of about 114 kDa [8]. It is an annular (ring-shaped), homo-pentameric protein which belongs to the pentraxin family. Physiologically, it binds to lyso-phosphatidyl choline which gets expressed on the surface of dead or dying cells (and some types of bacteria) to facilitate activation of the complement system and thus plays an important role in host defence as a pro-inflammatory mediator [9]. Elevated levels of CRP has been observed in infectious and in non-infectious inflammatory conditions. CRP serves as pro-inflammatory mediator by protecting the host system and hence CRP levels are considered as non-specific indicator of inflammatory activity, irrespective of the cause [10]. Since, a number of studies have investigated the relationship between pulmonary silica burden and the subsequent development of silicosis. But to the best of our knowledge, the promising biomarker for the early detection of silicosis has not been developed till date with special reference to Indian population. Hence, there is a need to pursuit a biomarker which can be effective in its early diagnosis. The aim of the present study was based on the above criteria, considering the role of CRP as an effective biomarker for early diagnosis of toxicity of stone quarry dust.

MATERIALS AND METHODS

Selection of area and subjects:

Stone quarries from Central India were selected for the study. A standard questionnaire was used to record information on base line characteristics such as age, sex; habits like smoking, tobacco chewing, alcohol consumption, duration of exposure to stone quarry dust, health history and medication etc. The study was explained to each volunteer and consent was taken from each of them. The study was approved by local Research Advisory Committee (RAC) and Institutional Ethics Committee (IEC).

Inclusion and Exclusion criteria:

Workers having exposure period more than 8 years were included in this study and those who were occupationally exposed to any known chemical agents, taking antacids, history of chronic diseases such as neurological disorders, cardiovascular diseases, diabetes, anaemia etc were excluded from the study. Total 209 subjects were selected for this study. These were further categorized as Experimental (Exp) and Control (Con). Subjects who were directly exposed to stone quarry dust were categorized into Exp group (n=101), for comparison, age matched healthy subjects, residing at the same geographical region but not exposed to stone quarry dust were selected as Con (n=108).

Blood collection:

Blood samples were collected from subjects in the medical room under dust free condition. To minimize the possibility of sample contamination, workers were instructed to report for the collection before the start of the shift. Total 5mL metal free, sterile syringes were used for the collection of blood. It was distributed as follows:

Total 5mL blood was collected among which 1mL blood was collected in sodium fluoride (100ug/mL) tube for blood glucose analysis. Remaining blood samples were allowed to clot and centrifuged at 1000 rpm for 5min. The resulting serum samples were transferred to sterile tubes. Three to four aliquots of serum samples were prepared and allowed to freeze. Samples were stored at -40ºC in accordance with accepted procedures. Serum samples were used for further analysis.

Determination of Biochemical parameters:

Among biochemical parameters Random Blood Sugar (RBS) and Liver Function Test (LFT) parameters were determined. RBS was analysed by GOD-POD method using commercially available kits (i.e. Beacon Diagnostics) and absorbance was measured at 505nm. Enzyme activity of Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetate Transaminase (SGOT) was measured in the sera by commercially available kit from Beacon Diagnostics. The value of total bilirubin and Alkaline Phosphatase (ALP) was determined by commercially available kits i.e. Precision Biotech and ENZOPAK-Reckon Diagnostics Pvt. Ltd respectively. The absorbance was measured at 340nm, 340nm, 540nm and 405nm respectively for SGPT, SGOT, Total bilirubin and ALP. The protein concentration of the serum sample was determined by using the Biuret method (Biosystem kit) following the manufacturer's instructions. The absorbance was measured at 545nm. Each sample was tested in duplicate.

Determination of CRP (ELISA)

CRP was evaluated by direct sandwich ELISA in the serum samples of subjects (Kit-SIGMA-ALDRICH, USA Cat log No. SE120041). Absorbance was measured at 450nm using ELISA plate reader (Systronics). Each sample was tested in duplicate.

Statistical Analysis:

Statistical analysis was performed using MedCalc Software *Version 10.1.2*. Chi square test and t-test of independent samples were used to evaluate the results for demographic parameters and years of exposure, CRP, biochemical parameters. $P<0.05$ was considered as significant. Pearson's correlation coefficient graphs of respective data were prepared using MedCalc software *Version 10.1.2*.

RESULTS

The study was conducted on stone quarry workers of Central India. Table 1 and 2 provides the descriptive statistics of basic characteristics of subjects in both the study groups and table 3 to 6 depicts the same information for males and females. As regards age, the difference in the mean age across study groups was found to be statistically insignificant (p= 0.136 i.e. *P*-value >0.05) by *t test for independent samples*. The mean age of subjects in Con group (38.71±4.94years) was close to Exp subjects (37.65±5.31years). The same trend was seen among males and females. It was observed that the mean age of Con was slightly higher than Exp in both the genders although the difference was insignificant as shown in table 3 and 5. The mean duration of exposure for subjects in Exp was found to be 17.77 ±5.61years (yrs). The duration of exposure was statistically significant between males and females (19.01±5.56 & 15.42±4.96 years) with *P value* of 0.0019 (*P value* <0.05) as per *t-test of independent samples.*

Fig 1 depicts the distribution of Exp subjects based on exposure to stone quarry dust. For convenience, frequency bar was created in the difference of 5yrs. Since, the minimum exposure period was 8 yrs, it was included under 6-10yrs of exposure. It is clear from the fig that there were seven males and seven females who had 6-10yrs of exposure to stone quarry dust. Nine male workers had highest duration of dust exposure in the range of 26-30yrs. However, among females, 6 workers had 21-25yrs of exposure. Apart from this, 19 workers among males and 13 among females were respectively having 21-25 and 11-15yrs of dust exposure. Tables 2, 4 and 6 present the mean and standard deviation of various biochemical parameters and CRP biomarker according to study groups. The statistical significance of difference in the overall mean values of parameters across study groups was evaluated using *t-test for independent samples*. It was observed that majority of the parameters differed significantly in both the groups (all with *P*-value < 0.05). The significance (for majority of the parameters) may be contributed by the mean levels of Con group. Statistically insignificant difference in both the groups was observed in case of few LFT parameters as evident from table. The same trend was observed among male and female subjects of both the groups. Conversely, females of both the groups also showed statistically insignificant difference in case of direct bilirubin. Significantly higher level of mean serum CRP was reported among Exp as compared to Con in all the cases. However, the value of CRP lies within normal range. This study indicates involvement of inflammatory reactions during the process of stone quarrying among exposed subjects.

Table 7 depicts the details of behavioral parameters according to study groups. Among behavioral habits, smoking (Smok.) and alcohol consumption (Alc.) showed insignificant association with the study groups while tobacco chewing (Tob.) was found to be significant using *Chi-square test* as shown in Table. The proportion of subjects with smoking habit in Exp group (48.51%) was insignificantly higher than Con (38.89%) group as revealed by *P*-value of 0.16 ($P > 0.05$). The same trend was observed in subjects having alcohol consuming habits with *P value* of 0.07 (P >0.05). In this case also the proportion of alcohol consuming habits was higher among Exp subjects (47.52%) compared to Con (35.19%). As regards tobacco consumption, significant difference was noted in both the groups with *P value* of 0.000 (P <0.05) using *Chi-square test*. However, the proportion of subjects having tobacco chewing habits was higher among Exp (70.30%) compared to Con (34.26%) as per the table. The same trend was observed among males and females of Exp and Con subjects. It is clear from the Table 7 that the behavioral habits were higher among male population compared to females. *Chi square* results showed no significant difference among males consuming alcohol but the difference was significant in case of smokers and tobacco chewers (*P value* 0.038 and 0.009 respectively). Among females, significance was only noted in case of tobacco chewing habits (*P value* <0.0001) as 21.78% females were tobacco chewers in Exp group compared to Con while no significant difference was noted in case of smoking and alcohol consumption habits.

Fig 2 explains the percent wise distribution of behavioral habits in both the groups. The table explains only the percentage of individual having any of the above habits *in toto*, however, bar chart depicts more clear information about the percentage of individuals having all these habits simultaneously or in

combination of any of the two or one. It is clear that Exp subjects had the highest percent of individuals having all the three habits simultaneously compared to Con when overall populations were taken into account. Highest numbers of tobacco chewers, Smok+Alc and Alc+Tob were also found among Exp subjects than Con. Further, 41.67% of individuals were found among Con who didn't have any habits which was highest than Exp subjects.

Male and female populations were also classified based on behavioral habits. It is clear from the fig 2 that subjects having all the three habits cover larger population among Exp males (i.e. 33.33%) than Con males (24.29%), whereas no female from both the groups were having all the three habits simultaneously. Moreover, 21.21% Exp males had both Smok+Alc habits which is further more than Con males (i.e. 11.43%). It was also noted that majority of the Exp females were either tobacco chewers (54.29%) or didn't have any habits (34.29%), while in case of Con females, most of them were not having any behavioral habits (89.47%). Only 3.03% Exp males were not having any habit which is very less than Con males (15.71%).

It has been reported that smoking habits influence the level of CRP. The effect of behavioral parameters on CRP level was evaluated and shown in table 8 and 9 for Con and Exp subjects respectively. It is evident from the table 8 & 9 and fig 3 that subjects who had only smoking habit was having high CRP values. Since, tobacco also contains nicotine, the combined effect of duo of these habits increases CRP levels among Exp subjects while the same trend was not observed among Con.

Figure 4 (a) shows the Pearson's Correlation Coefficient between serum CRP levels and age of subjects of both the groups. Insignificant positive correlation (r= 0.077, p =0.439 i.e. *P valu*e >0.05) was observed between both the parameters among Exp while in case of Con no correlation was observed with insignificant *P value* 0.70 (P= >0.05).

Figure 4 (b) shows the Pearson's Correlation Coefficient between serum CRP levels and years of exposure to stone quarry dust. Significant positive correlation was observed between years of exposure to stone quarry dust and CRP (r= 0.20, p =0.04 i.e. *P valu*e <0.05). Since, Con subjects didn't have exposure; correlation could not be drawn for this subjects.

Fig 4 (c) to 4 (f) respectively shows relationship of LFT parameters with CRP among both the subjects. Insignificant correlation was obtained among all the parameters between the two in case of Exp while as far as Con subjects is concerned, except Total bilirubin and SGOT, other parameters were found to be insignificant. Inverse Pearson's Coefficient Correlation was obtained for Total bilirubin and SGPT with CRP in case of Exp and Con subjects. It was r= -0.08 with *P value* 0.403 (P= >0.05) and r= -0.21 with p value 0.02 (P= <0.05), for total bilirubin Vs CRP among Exp. and Con respectively while r= -0.08 with *P value* 0.414 (P= >0.05) and -0.04 with p value 0.668 (P= >0.05) for SGPT Vs CRP among Exp. and Con respectively. Similarly, insignificant positive correlation (r= 0.02, p= 0.796 and r= 0.03, p=0.694) was obtained for ALP Vs CRP among Exp. and Con respectively whereas positive correlation (r= 0.05, p=0.584) and negative correlation (r= -0.20, p=0.034) was obtained for SGOT Vs CRP among Exp. and Con respectively.

DISCUSSION

The present study was focused on role of CRP biomarker among stone quarry dust exposed miners, in pursuit of its' potentiality to be a signature biomarker of Silicosis and other occupational diseases. Limited literature is available on estimation of CRP levels in various other diseases but to the best of our knowledge, in stone quarry dust exposed miners its level is not yet measured especially in Indian population. The results of the current study showed that the mean age among Con (38.71±4.94yrs) was close to Exp subjects (37.65±5.31yrs). Age plays a critical role in development of risk of silicosis among exposed population than control because as the age increases the duration of exposure to dust also increases. Since, the mean duration of exposure to dust was found to be 17.77 ±5.61yrs. Further, the minimum duration of exposure was 8 years and as cleared from the fig 1 that there were seven males and seven females who had 8-10yrs of exposure to stone quarry dust. Nine male workers had highest duration of dust exposure in the range of 26-30yrs. However, among females, 6 workers had 21-25yrs of exposure. Apart from this, 19 workers among males and 13 among females were respectively having 21-25 and 11-15yrs of dust exposure. Zawilla N. *et.al.* [11] included Brick dust (that contains silica) exposed workers in his study, who had minimum exposure of 5yrs and found that half population had silicosis. He opined that exposure to silica dust suppresses immunity at first but persistent exposure to silica dust elicits immune response. Our finding is in consistent with the findings of above reported study as in the present study workers having minimum of eight years of exposure were included. Therefore, there lies strong possibility of development of silicosis in our subjects. The insignificant positive correlation between CRP and age in case of Exp subjects indicates increase in CRP level with age, however no correlation was observed between the duos in case of Con. The insignificant positive Pearson's

Correlation Coefficient between serum CRP levels and years of exposure to stone quarry dust among Exp subjects suggests that as the duration of exposure to stone quarry dust increases, CRP level also gets increases. Since, the mean CRP level was found to be within normal range but as per Kalliny MS., Bassyouni MI., (2011) higher levels of CRP are indicative of inflammatory condition [12].

Since smoking and alcohol consumption habits were higher among Exp group than Con; especially, the percentage of male smokers were more compared to female as far as Exp group is concerned; the risk of development of silicosis and ultimately silicosis linked lung cancer may be more among these population. The percentage of smokers and alcohol consumers among Con was also not negligible but these habits along with exposure to silica dust pose a considerable risk among Exp subjects. This finding is in line with Sivanmani K., Rajathinakar V., (2013) who documented that subjects having smoking habits along with silicosis had higher risk of Cancer in future [13]. As regards tobacco consumption, higher percentage was noted among Exp subjects compared to Con. Exp male population had the highest number of subjects consuming tobacco than Con and females of both the groups. The risk of development of silicosis and other occupational diseases may be higher among these populations. It is clear that Exp subjects had the highest number of subjects who preferred combination of behavioral habits (i.e. Smok+Alc, or Alc+ Tob or Tob +Smok) compared to having a single habit (i.e. either of them). The reverse trend was observed among Con subjects. The association of development of silicosis may be linked with presence of nicotine compound in tobacco and also in smoke, which elicits the Cell Mediated Immunity (CMI) response of the body and hence directly and/or indirectly helps in patho-physiology of silicosis. Among liver function test parameters, except bilirubin and SGOT, all other parameters were insignificant. Total bilirubin, indirect bilirubin, SGOT, SGPT, and ALP were found to be higher among Exp subjects compared to Con. The same trend was observed among males and females of Exp subjects than Con. However, it falls under normal range. These observations are in consistent with Ilahi I. *et. al.* (2012) who found significantly enhance levels of bilirubin, SGPT, Alk PO_4 among stone dust exposed populations compared to control in Pakistan [14]. Significant rise in total protein levels was noted by Saxena S. [15] after sub-chronic exposure of silica to animal models compared to control. However, in the present study, no significant difference was noted between Exp and Con with regards to total protein level and it falls under normal range. Exp males and females showed the same phenomenon. Ilahi I. *et. al.* [14] reported that toxic compounds of stone quarry dust get transpose from the lungs to other body tissues and organs, including liver. Hence, it can be said that Exp population may have dysfunctioned liver attributed due to prolonged exposure to stone quarry silica dust [16]. Inverse Pearson's Coefficient Correlation for total bilirubin and SGPT with CRP in case of Exp and Con subjects and positive Pearson's Coefficient Correlation (although insignificant) for ALP with CRP in case of Exp and Con subjects indicate the alteration of liver function in response to dust exposure which in turn elicits the ALP and CRP level among Exp. This finding is in accordance with the results obtained by Kerner A. *et. al.* [17] who worked on workers having metabolic syndrome and the role of hepatic contribution in increasing systemic inflammation. He found that increase in liver enzyme levels are linked with enhanced CRP concentrations. C Reactive Protein (CRP) was taken as the marker to determine toxicity of stone quarry dust among exposed workers. Since, the findings of CRP were within limit in both the groups but it was significantly higher among Exp subjects compared to Con. The same finding was noted among males and females of Exp subjects than Con. Additionally its value was found to be higher in subjects having only smoking habits in case of both the groups. It is in accordance with reported study stating that CRP level increases in autoimmune diseases and active smokers. This finding is in relative resemblance with study conducted by Kalliny MS, Bassyouni [12] who worked on effect of free crystalline silica dust exposure in Egyptian Phosphate Mine worker. Moreover, 48.5% of Exp subjects were smokers (*in toto*). The combined effect of tobacco consumption and smoking to increase the CRP level in case of Exp but not in Con could be explained by the increased immunological response against inhaled stone quarry dust. High CRP levels augur the involvement of inflammatory reactions among Exp subjects compared to Con which is further supported by the finding of LFT parameters.

CONCLUSION

On the basis of the findings of our study, it is postulated that exposure to stone quarry dust alters biochemical profiles causing high level of serum CRP. Exposure to dust more than 8 years is responsible for initiation of occupational diseases. Determination of serum CRP levels along with LFT parameters may give evidence about the severity of disease. Stone quarry workers who are active smokers are at greater risk of development of occupational diseases especially silicosis. Male workers are more prone to occupational illness than females who are working in stone quarries. These study may be helpful for other researchers as base study among stone quarry exposed Indian population; and CRP could be used as supportive biomarker for early detection of health risks in workers exposed to stone quarry dusts. Further investigation with large number of sample is needed to confirm the findings of the present study.

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