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RESEARCH ARTICLE

Rapid Growth of *Mycobacterium* Species in Patients with Occupation Related to Silica

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ABSTRACT

In this study sputum culture positive cases for tuberculosis in automated TB culture system were studied for patients with occupation related to silica and for patients with occupation not related to silica and their important related findings were categorized and analysed. Similarly control groups were also studied who were TB culture negative in automated TB culture system. Tuberculosis was found common in the age group of 21-30 years, but in patients with silica related occupation, it also occurred at a higher age group and in silica group there is a male preponderance. Among associated microorganisms *Candida* spp. were found more in silica group than in non-silica group. The growth pattern of *Mycobacteria* spp. Indicated a definite role of silicon on multiplication and growth of *Mycobacterium* spp.

KEY WORDS: Mycobacteria, silica, bacterial growth.

INTRODUCTION

Many organisms are known to be able to utilise silicon (Si) which is present plenty in nature. There are distinct Si accumulator plants like Cyperaceae, Graminae, Juncaceae and Moquiles Spp.; organisms like marine phytoplankton, marine brown algae, 'horsetails', foraminifera and porifera contain enough Si, in the range of 60,000-4,37,000 mg per kg dry matter (DM). Bacteria contain about 180 mg per Kg DM of Si[1-2]. Addition of Si in culture media showed a remarkable growth accelerating effect on *Staphylococcus aureus*[3] including its utilisation[4] and in Nocardioform organisms[5]. There is considerable indirect evidence of Si utilisation by bacteria. Thus, 'non-typhoidal Salmonella', *Enterobacter*, *Klebsiella* and *Citrobacter* can survive better in Si-riched sandy-loam soil than in the plain loam soil[6]. There is a profound similarity of Si chemistry and carbon (C) chemistry[7]. The silicon compounds have kinetic parameters identical to those of their carbon analogues[8]. It is possible that an organism can utilise Si in a C-deficient environment. Silicon is the second most abundant element in the lithosphere (27.70%) and it is as important as phosphorus and magnesium (0.03%) in the biota [9] Hydrated silica represents the second most abundant biogenic mineral after carbonate minerals [10] Silicon is accumulated and metabolized by some prokaryotes [11], and Si compounds can stimulate the growth of a range of fungi [12] It is well known that Si is essential for diatoms [13] In mammals, Si is considered an essential trace element, required in bone, cartilage and connective tissue formation, enzymatic activities and other metabolic Processes[14-16] Silicon was suggested to act as a phosphoprotein effector in bone [17]. In mammals, Si is also reported to positively influence the immune system and to be required for lymphocyte proliferation [18]. The aqueous chemistry of Si is dominated by silicic acid at biological pH ranges [19]. Monosilicic acid can form stable complexes with organic hydroxy-containing molecules [20] . Biosilica also has been identified associated with various biomolecules including proteins and carbohydrates [21]. Hypervalent forms of silicon have been found to complex with a range of sugars and sugar derivatives [22,23]. Recently, Kinrade *et al*. [24] reported the first evidence of an organosilicon compound formed in vivo in the diatom *Navicula pelliculosa*. In diatoms, Si was suggested to affect phosphorylation of specific proteins required for the synthesis of DNA and specific mRNA [25,26]. In this paper sputum culture positive cases for tuberculosis in automated TB culture system were studied for patients with occupation related to silica and for patients with occupation not related to silica and their important related findings were categorized and analysed. Similarly one control group was also studied who were TB culture negative in automated TB culture system which is a sensitive test for detection of tuberculosis.

MATERIALS AND METHODS:

100 patients were selected in each group (with) occupation related to silica and with occupation not related to silica and with suspected tuberculosis by the clinicians) and they were instructed to collect sputum for three consecutive days as per WHO protocol. With collected sputum smear studies and culture studies were done in automated culture systems. A routine culture

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examination was also done to find out associated organisms in the sputum.

RESULTS:

AGE DISTRIBUTION IN DIFFERENT GROUP:

In general sputum TB culture positive cases peak age group is 21-30 years, however, if we differentiate silica group and non-silica group then we find that in silica group although peak age group is the same but there is a tendency of biphasic appearance with another minor peak at age group 41-50 years. In non-silica group a general pattern is present. This age group pattern predominance may indicate that tuberculosis is common in the age group of 21-30 years; however, in patients with silica related occupation, it also may occur at a higher age group. Thus in a population there is prevalence of at least two genetic loci for tuberculosis one of which is related to silicosis and/or silico-tuberculosis.

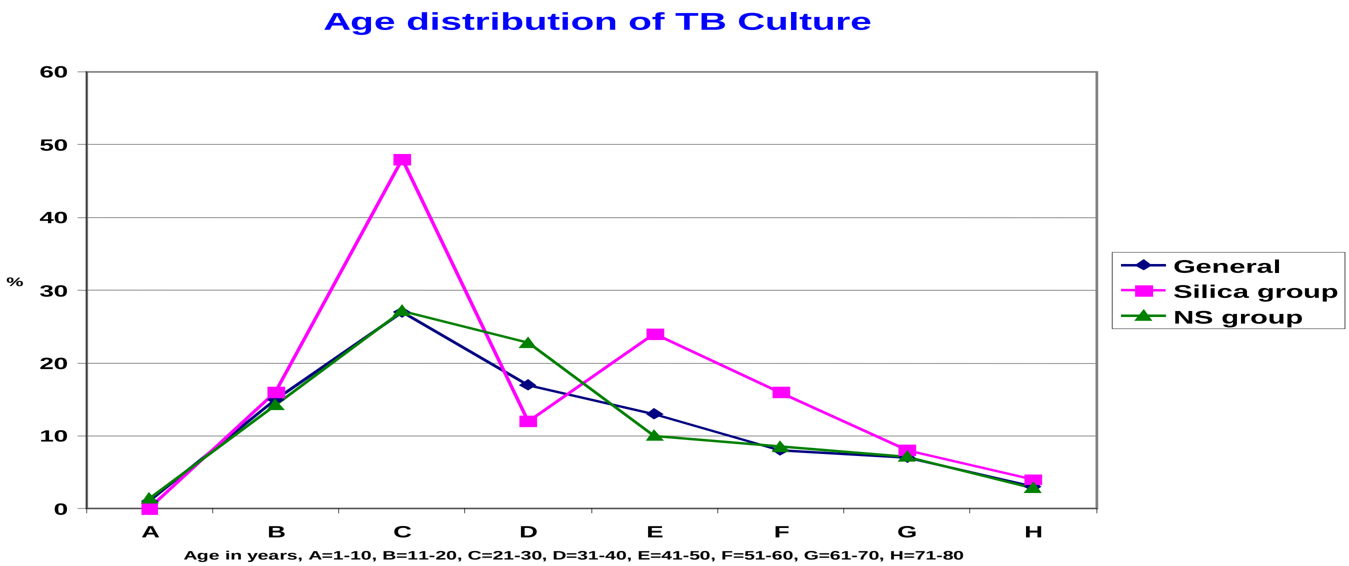


Figure no 1: age distribution of T B culture

SEX DISTRIBUTION:

Although males were found affected more in tuberculosis, but in silica group males are almost exclusively affected (93% in silica group, 55.7% in non-silica group.). However, in non-tuberculous cases males were also more in number (58%). Thus in silica group there is definitely increase of male preponderance. This is probably due to their occupation in relation to silica.

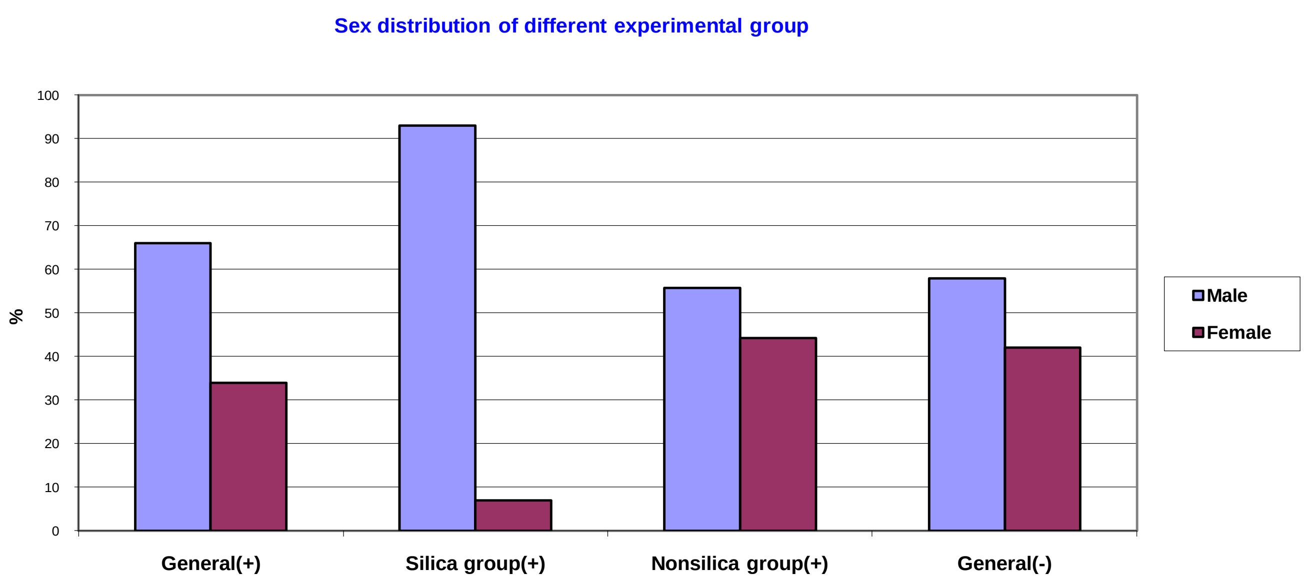


Figure no2: sex distribution of different experimental group

CULTURE POSITIVITY:

In culture positive cases which were shown positive in automated TB culture system, 30% were negative in routine smear examination, which is very significant. Thus this indicates that by routine smear examination we may miss about one third of TB cases. Another important point to note is that in silica group degree of positivity is significantly more in comparison to silica negative group or in comparison to the general positve cases. Thus it was found that in silica group 3+ positive cases were 50%, on the other hand this was only 23% cases in general cases and only 14.28% cases in group not related to silica. This finding indicates that silica has definitely have a role in multiplication and pathogenesis of *Mycobacterium* spp.

TIME REQUIRED FOR A POSITIVE CULTURE:

Similarly when time required for a positive culture in an automated system then again it was found that in silica group time required for a positive culture was significantly less in comparison to the non-silica group or the general group. This again indicates a growth promoting role of silica on *Mycobacterium* spp.

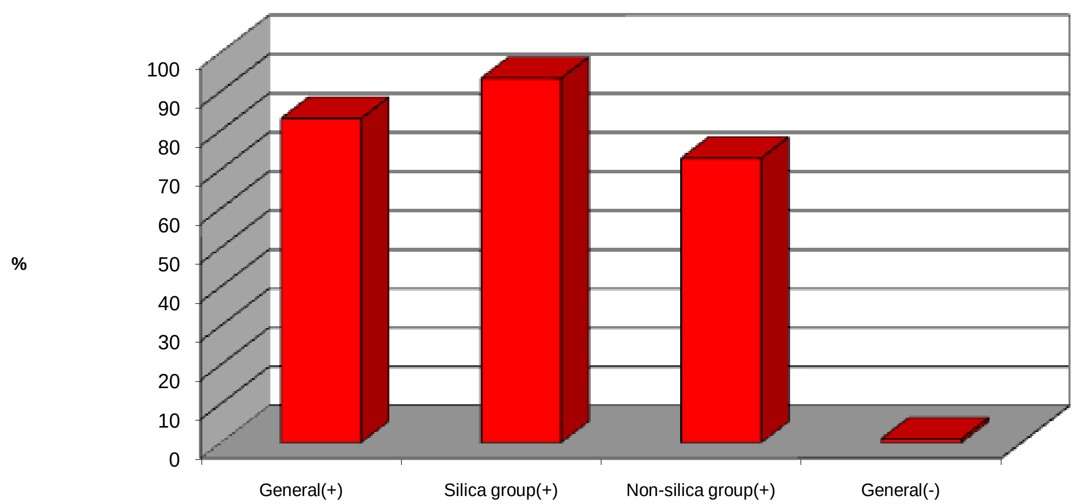
IDENTIFICATION OF *MYCOBACTERIUM* SPP.

There were no significant differences of the identification of the *Mycobacterium* spp. in all the groups. Thus this may indicate that pathogenic *Mycobacterium* spp. which is prevalent in a locality can cause infection according to their prevalence and this is not dependent on the presence or absence of silica.

ASSOCIATED MICROORGANISMS:

If we consider the common associated microorganisms, which were found associated with TB infection, then it is very interesting to note that *Candida* spp. were found commonly in sputum of TB positive cases (83%) in comparison to TB negative cases (only 1%). *Candida* spp. were also found more in silica group than in non-silica group. Although this finding is important to note with but this is very difficult to explain. A possible explanation of this finding is excessive use of usual antibiotics in these cases before their diagnosis of tuberculosis. Excessive use of antibiotics usually suppresses the normal flora and allows *Candida* spp. to increase in significant numbers where they usually normally do not present. Many other microorganisms were also found in TB positive and negative cases. However, there was no significant difference of incidence of these organisms.

Isolation of Candida spp.



DISCUSSION:

The results of this study clearly indicate that there is a definite role of silicon on multiplication and growth of *Mycobacterium* spp. However, it is difficult to explain how it elicits such activities in this preliminary study.

Silicic acid may modulate the activity of post-elicitation intracellular signaling systems. Hutcheson[27] has distinguished several classes of active non-tuberculous defense mechanisms. The primary response occurs in cells infected by the pathogen, elicitors induce the secondary response and limited to cells adjacent to the initial infection site, and the systemic acquired response is transmitted hormonally to all tissues. Silicon is perhaps acting in the primary response, and the integration of enhanced signal transduction at the single cell level should result in increased levels of induced systemic resistance. Post-elicitation intracellular signaling leads to the expression of defense genes directing hypersensitive response, structural modifications of cell walls, stress hormones synthesis, antimicrobial compounds synthesis and PR proteins. As mentioned earlier, Si is involved in the processes leading, among other responses, to the accumulation of alexins. The target of signaling upon pathogen elicitation is the cell nucleus, which receives information for de novo protein and antimicrobial compounds synthesis. Gene expression control through the phosphorylation of transcription factors and their inhibitors is a major stress response. Signals leading to the expression of defense responses are transmitted to the nucleus through the activation of specific kinases/phosphatases cascades. This can be generalized to both endogenous [28-33] and exogenous [34] signaling events. Responses to biotic stresses are largely dependent on mitogen activated protein (MAP) kinases [35-38]. Protein kinases transmit information to the nucleus by the phosphorylation of hydroxyl group on amino acid residues. Silicon is known to bind to hydroxyl groups and may thus affect protein activity or conformation. The mode of action of Si in signal transduction may also derive from interactions with phosphorus. As early as 1906, Hall and Morrison[39] reported interactions between Si and phosphorus. It is now considered that the internal improvement of P utilization and the broadening of P fertilization range provided by Si fertilization [40] derives from interactions with cationic metals such asMn and Fe [41]. Metals play a structural role for many enzymes. Enzymatic dysfunctions may derive from the excess of essential metal species or the presence of toxic metal species [42]. Whether Si improves defenses indirectly by sequestering cationic metals, or directly by modulating protein activity involved in signal transduction remains to be investigated. Upon pathogen attack, the infected tissue will synthesize, among other defense reactions, antimicrobial compounds together with systemicstress signals such as salicylic acid, jasmonic acid and ethylene. In a given cell, if Si indeed modulates the signaling events leading to the synthesis of nontuberculous antimicrobial compounds, it should also modulate the generation of systemic signals given that both processes depend on primary elicitation. Accordingly, silicic acid, without being itself a secondary messenger, could play a positive role in both local and systemic non-tuberculous resistance. Thus all these effects indicates that although there are systems that silica can protect body from ordinary bacterial infections which were suppressed in presence of silica, allowing more growth of *Candida* spp. and *Mycobacteria* spp. in all patients with silica related occupation.

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