Silica particles as a possible actiological factor of esophageal carcinoma in the Qassim region of Saudi Arabia; A pilot study.

Ammar C. Al-Rikabi, MRC(Path), Mohammad O. Al-Sohaibani, FCAP, Mahmoud H. Ashour, FRCS, Saleem M. Baig, MSc.

ABSTRACT

Objective: To determine the causative relation between the high levels of silica content in local wheat and salt and the reported increased incidence of esophageal carcinoma in the Qassim region of Saudi Arabia. [1-4] **Design:** The wheat and natural salt produced in the Qassim province of Saudi Arabia are analyzed using the "ash weight method" in order to determine their content of silica. At the same time, the acid insoluble particles of silica isolated from esophageal squamous epithelium surrounding the tumor-bearing site are assessed using a filtration method for extraction. The particles are subsequently observed by a scanning electron microscope and the elements measured by an x-ray analyzer. The results obtained from the experimental and control specimens are tabulated and compared. **Subjects:** The resected esophageal segments of four Saudi patients who were diagnosed to have esophageal carcinoma and have been resident in the Qassim province formed test group number I (group I). Group II (the control tissue specimen) consisted of equal weight of mucosal samples obtained from four normal esophageal segments taken during necropsies performed in Riyadh Central Hospital. **Results:** The results of this retrospective pilot study showed that the wheat and natural salt produced in the Qassim province contain significantly higher levels of silica when compared with that of other countries like United Arab Emirates and Australia: 130 (6.5%), 74 (3.7%) and 34 mg (1.7%) of silica per 2000 mg of wheat respectively and 14 mg (0.12%) only of silica per 2000 mg of sample. A comparison between the mean values of the counts and weight of silica granules in carcinomatous esophageal specimens and that of control samples show a significant increase in the levels of silica in the resected esophageal segments. Table 1. **Conclusions:** This pilot study suggests a previously unconsidered possible actiological relation between the significant silica contents in the wheat and salt and the increased reported incidence of oesophageal carcinoma among the local population of Qassim's province. It is possible that the purative sharp-edged silica particles cause repeated local injury during their passage down the esophagus and also during the time they spend buried in the mucosa. This repeated injury may lead to cellular proliferation and subsequent neoplastic change.

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A high content of silica particles was found in the millet bran which is a component of the diet in the area of highest esophageal cancer incidence in Northern China.[5] High levels of silica fragments and fibres were also detected in the diet of two other regions of greatest incidence of esophageal cancer, the Transkei in South Africa[6] and Iran.[7] A high incidence of oesophageal cancer has been reported in the Qassim region[1-4] which is an agricultural area in the central province of Saudi Arabia. Life style, dietary habits and environmental conditions may play a dominant role in the incidence of oesophageal carcinoma.[3,4] It was also hypothesized in a previous study[1] that the high incidence of esophageal carcinoma in the Qassim region could be due to contamination of underground water with petroleum products. The aim of this pilot study was to assess the possible role of silica in the pathogenesis of oesophageal carcinoma in the Qassim province. A two limb study was conducted and the results and discussion of our investigations form the basis of this paper.

Materials and methods. ***Extraction of acid insoluble particles from wheat and salt.*** The weight of silica content of five separate two gram samples of wheat grains obtained from the Qassim farms, as well as equal weights of identical Australian and United Arab Emirates wheat were measured using the Ash-method. Equal samples of local and imported food salts were also assessed for weight of silica content using the same method. The samples were incinerated and the residue dissolved in diluted nitric acid. The samples were then washed with water and filtrated using a pre-weighted cellulose nitrate filter (size of pores 0.2 µm). The differences in the weight of the filter represented the weight of the silica content. For the

From the Department of Histopathology (Al-Rikabi, Al-Sohaibani), Division of Electron Microscopy (Baig), and Thoracic Surgery (Ashour), King Khalid University Hospital, Riyadh.

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Address correspondence and reprint request to: Dr. Ammar C. Al-Rikabi, Department of Pathology (32), King Khalid University Hospital, PO Box 2925, Riyadh 11461, Kingdom of Saudi Arabia. Fax No. 467 2462.

Table 1 Weight and percentage of silica particles in 2 gram samples of Qassim (GQ), UAE (GUAE) and Australian (GAU) wheat and equal weights of local and imported salts.

Type and source of samples	Percentage (%) of silica content	Weight of silica (SiO_2) content per 2000 mg sample
GQ	6.5	130 mg
GUAE	3.7	74 mg
GAU	1.7	34 mg
Food salt (Qassim)	0.7	14 mg
Food salt (elsewhere)	0.12	2.4 mg

Table 2 - Weight of acid-insoluble granules in esophageal tissue obtained from patients and controls. This table also shows the ratio between weight of granules in the mucosa surrounding tumor and total weight of tissue per specimen.

Experimental (set I) & control specimens (set II)	Wt. of normal mucosa	Wt. of granules in normal mucosa	Wt. of granules in mucosa surrounding tumor	Ratio–Wt. of granules in muc. surr. tum. / Total wt. of Tis.
Sps. (1)	3000 mg	22 mg	30 mg	0.01
Sps. (2)	3000 mg	20 mg	35 mg	0.012
Sps. (3)	3000 mg	25 mg	30 mg	0.01
Sps. (4)	3000 mg	34 mg	40 mg	0.013
Control (1)	3000 mg	10 mg	-	-
Control (2)	3000 mg	15 mg	-	-
Control (3)	3000 mg	15 mg	-	-
Control (4)	3000 mg	15 mg	-	-

food salt samples, the silica content was determined after dissolving two grams of five different samples in water. The solution was then washed with diluted nitric acid and filtrated using a previously weighted cellulose filter paper.

Silica particles in esophageal tissues. In parallel with the above study, estimation of silica particles in esophageal tissues was conducted in two different sets of esophageal segments. In set I (test group I), 3000 mg of esophageal mucosa were obtained from the mucosa surrounding the tumor bearing area of four esophageal segments which were resected because of esophageal carcinoma. In set II (control group), equal weight of esophageal mucosa was obtained from four normal esophageal segments taken from necropsies performed on subjects not known to have oesophageal carcinoma.

To isolate the acid-insoluble material, the experimental group (set I) and control group (set II) samples were then cut into small pieces with a scalpel blade and placed in 50 ml of Kjeldahl flasks. Twenty ml of fuming nitric acid was added to the flasks and shaken well. After the reaction had subsided, the mixture was heated to boiling point and refluxed for 6 hours. The residues were suspended in 200 ml of double distilled and deionized water and passed through a 25 mm cellulose nitrate filter (size of pores-0.2 µm). The filters used were thoroughly washed with water, dried under cover and weighed. Filters prepared in this way could be prepared for electron microscopy. After filtration, the cellulose membranes were re weighed and the differences between the weights considered as the weight of acid-insoluble granules.

Dimensional, scanning electron microscopy and x-ray analysis of particles. The cellulose membranes prepared above were put on special stubs and examined in a Jeol 35c scanning electron microscope (SEM) using an accelerating voltage of 25 KeV, and a take-off angle of 30.3°. The beam current was 3 x 10^{-9}A. An energy dispersive x-ray detector (Oxford ISIS-microanalysers system) fitted with an atmospheric thin window (light element EDS) was used to perform the analysis of the elements. Size distributions were determined on micrographs made with the ordinary secondary emission image. Tissues were examined with the x-ray distribution map which is specific for silicon. Only element with atomic numbers greater than 6 could be detected. These methods of elements observation and analysis were also described and used by other investigators. [5]

Results. ***Silica content in the diet of Qassim region.*** Five separate two gram samples of wheat from Qassim showed higher levels of silica particles (6.5%) when compared with equal weights of wheat from UAE and Australia (3.7%, 1.7%) respectively. Moreover, Qassim salt showed a higher level of silica (0.7%) when compared with an equal weight of commercial salt (0.12%). Acid-insoluble content of esophageal mucosa. The test group samples of esophageal tissue (set I) revealed a large

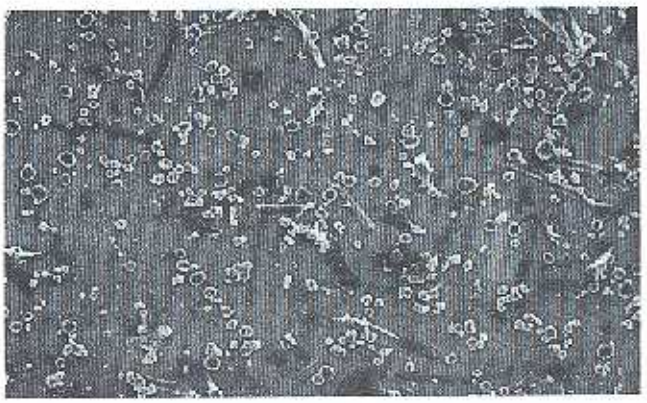

Fig. 1 SEM photomicrograph of the surface of 25 mm filter (area of active area: 20 mm) used to collect the residue from experimental esophageal mucosa. Note: the presence of massive residue of acid-insoluble particles.

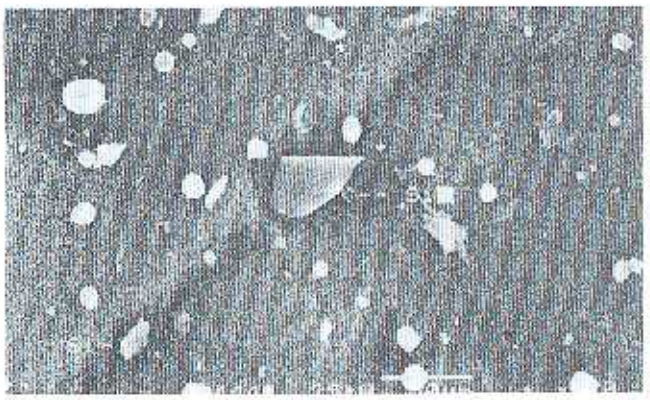

Fig. 2 SEM photomicrograph showing the area of acid insoluble particles which were subjected to elementary x-ray analysis

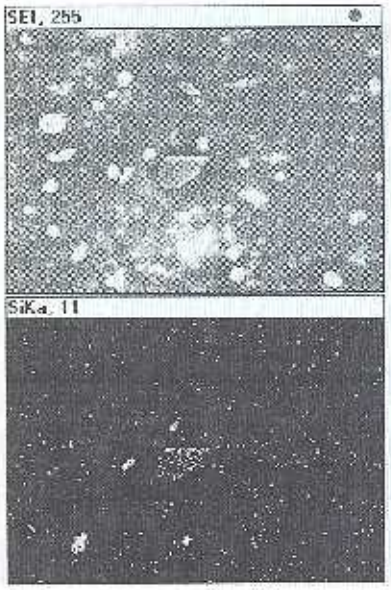

Fig. 3 - Top: SEM computer generated micrograph showing acid insoluble particles isolated from experimental tissue. Below: The same area was subjected to elementary x-ray analysis which enables the isolation of silicon particles only.

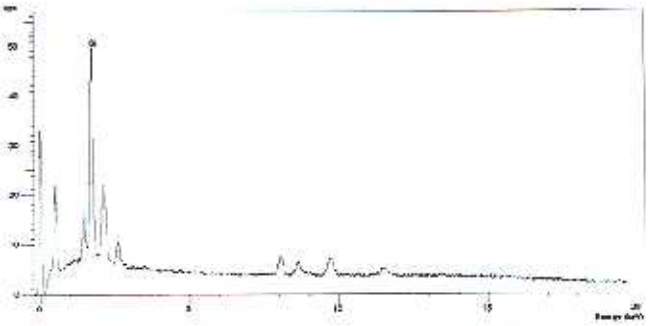

Fig. 4 - (A) - Graphical depiction of x-ray analysis of experimental esophageal tissue showing quantitative content of silicon (Si), aluminum (Al) to the left of Si peak, phosphorous (P) and chloride (Cl), consecutively to the right of Si peak. Note: the high content of silicon.

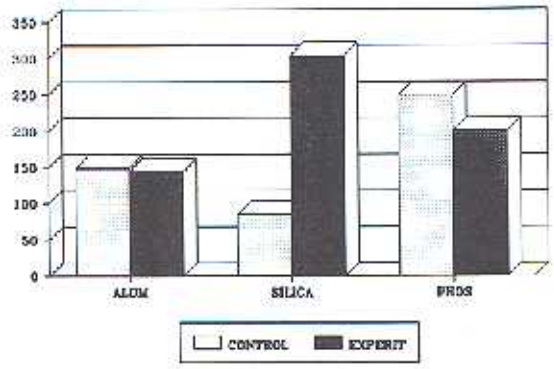

Fig. 5 - Graphical comparison between the quantities in mg/kg of aluminum, silicon particles and phosphorous obtained from experimental and control tissues. Note: the marked increase of silicon in experimental tissue.

amount of silica particles. A typical result is depicted in Figure 1. The weight of the acid insoluble particles in set I and set II esophageal mucosal samples were estimated by using the direct gravimetric method. The weight of acid insoluble granules in set I (mucosa surrounding tumor) were: 30, 35, 30 and 40 mgs respectively, while the weight of granules in set II (control samples) were 10, 15, 15, 15 mgs respectively, Table 2. The ratio between the calculated mean weight of acid-insoluble granules in patients (33.75 mg) and control specimens (13.7 mg) is equal to 2.45. This finding indicates the presence of approximately two and a half folds of acid insoluble particles in the test tissues (set I) than the control tissues (set II). The elemental composition of small representative samples taken from the test tissues were determined by an energy dispersive x-ray analyzer. The results obtained are depicted in (Figs. 2 and 3). The graphical analysis showed the presence of aluminum (Al), phosphorous (P), silicon (Si) and chloride (Cl) in various amounts (Fig. 4). The amount of silicon particles in set I esophageal samples was increased by a statistically significant amount when compared with set II (control samples) (Figs. 4 and 5).

Discussion. The rate of death from oesophageal cancer varies greatly in different countries and regions. Data obtained by the World Health Organization and published in 1977[1] showed that the mortality rate, standardized to the world population, was highest in both sexes in China, Puerto Rico ranked second and Singapore ranked third. Studies in the high-risk areas, such as the eastern coastal regions of the Caspian sea in Iran[2] and in Lixian, in China,[3] have suggested that dietary, environmental and endogenous factors are aetiologically important in esophageal cancer. In the high-risk areas of esophageal cancer, poor dietary conditions may play an important role. There is often a low intake of vitamin A and C, riboflavin, animal protein, fat, fresh vegetables and fruit.[3,10] Zheng and associates[10] studied the urinary excretion of a test dose (5 mg) of riboflavin in peasants of Linxian. Most of the peasants tested excreted less than 1 mg of riboflavin in the urine over a 4 hour period, which indicated inadequate intake of riboflavin. The role of trace elements has also been investigated in China and Transkei,[11] where the incidence of esophageal cancer is high. A high content of silica particles was found in the millet bran which is a component of the diet in the area of highest esophageal cancer incidence in Northern China.[3] High levels of silica fragments and fibres were also detected in the diet of two other regions of greatest incidence of oesophageal cancer, the Transkei in South Africa[9] and Iran.[7]

The Qassim region of Saudi Arabia is well known for its high incidence of esophageal cancer.[1-4] The wheat is cultivated on a large scale in the local farms and natural salt is obtained from certain mining areas. In this study, we found significant levels of acid-insoluble silica particles in both local wheat and salts using filtration and ash gravimetric-methods for extraction. Heavy deposition of silica particles was seen in the mucosa of resected esophageal segments obtained from patients with esophageal carcinoma. The nature of silica particles in these samples was confirmed and quantitated by using a scanning electron microscope and an energy dispersive x-ray analyser. The mechanism by which silica particles act

is not known, but repetitive trauma have been postulated.[12] It is possible that the silica fragments could induce a repeated local injury during their passage down the esophagus and also during whatever time they spend buried in the mucosa. Furthermore, this repeated injury may lead to DNA damage and point mutations with subsequent cellular proliferation and neoplastic change.

Further work is needed to test whether silica particles of a certain shape and size might be involved in the aetiology of esophageal cancer in the Qassim region. Other possible contributing aetiological factors such as dietary riboflavin, trace elements and nitrosamines[13,14] should also be considered and assessed. Genetic factors and familial aggregations as noted in Chinese and Iranian patients with oesophageal cancer[15,16] may also be relevant to cancer incidence in the Quassim community where marriage between relatives is a common custom. The authors are aware that this clinico-pathological study of a limited number of cases and controls should be regarded as a pilot study.

We do feel, however, that this work would constitute the basis for further similar studies on a substantially increased number of cases. The techniques used could also be applied on endoscopic esophageal biopsies. We hope that this study will help to draw the attention of the health authorities to the possible risks of using mined local salt in the domestic diet.

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