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MILK ALKALI SYNDROME:
AN OCCUPATIONAL DISORDER FOR CONSIDERATION

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ABSTRACT

Background: The milk alkali syndrome has always been suspected when patients present with hypercalcemia, renal impairment and metabolic alkalosis. Nowadays, the occupational exposure to carbonate salts at the workplace is considered an environmental problem that seems to increase the possibility of the appearance of more cases of the syndrome.

Objective: investigating the effects of exposure to lime stone dust among working population in the petrochemical industry and determining the importance of interpreting the unexplained abnormal kidney functions in the diagnosis of chronic form of milk alkali syndrome.

Methods: Detailed assessment questionnaire and thorough clinical examination were used to study the health condition of 24 workers exposed to sodium and calcium carbonate during the processing of limestone and of 21 matched non-exposed workers. The cardiovascular changes were further evaluated using the electrocardiogram. The blood gases as well as sodium, potassium and chloride were estimated by the automated analyzer. Laboratory tests investigated the serum levels of ionized calcium and phosphorus calorimetrically and the 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D)

by chromatography. As for the intact parathyroid hormone (PTH) and parathormone hormone related peptides (PTH-rP) in serum, estimation was carried out using the two-site immunoassay technique.

Results: Urinary frequency was detected among exposed workers as sign of isotonic polyuria caused by increased stimulation of calcium-sensitive receptors in the collecting tubules. Elevation of ionized calcium levels (mean values 8.32 ± 0.72 mg/dl) among exposed workers resulted in lowered glomerular filtration rate as evidenced by azotemia. A positive linear association was detected between urea and the ionized levels of calcium. At the renal tubules, hypercalcemia induced bicarbonate absorption that led to metabolic alkalosis as evidenced by elevation in blood pH (7.46 ± 0.03) and bicarbonate equivalent above 28 mEq/L, and by the positive correlation of calcium and PTH with blood gas parameters. The resulting decreased kidney activity appeared to be a major factor in derangement of the vitamin D metabolism and lowering of phosphorus levels. Deficiency of vitamin D stimulated the release of parathyroid hormone highly significantly to maintain calcium level via decreasing urinary excretion and increasing renal calcium re-absorption. PTH and calcium, which were positively associated together highly significantly, both showed negative correlations with the 1,25-$(OH)_2$-D level. The PTH-rP was significantly low among exposed workers excluding the possibility of any active role in the development of hypercalcemia. Age and smoking were not considered confounding factors in the study compared to the exposure duration which was significantly playing a positive role in the development of alkalemia and hypercalcemia.

Conclusion: The obtained data suggested the presence of a relationship between the exposure to limestone and renal affection. The milk alkali syndrome which was a familiar entity might therefore be considered an occupational disorder that is manifesting in an unfamiliar way. The important element in diagnosis is the initial consideration of milk alkali syndrome as potential cause of symptoms seen among workers with hypercalcemia.

Key words: calcium carbonate, limestone, soda ash, milk alkali syndrome, hypercalcemia, phosphorus, 1-25 dihydroxyvitamin D, intact parathyroid hormone, parathormone hormone related peptides, metabolic alkalosis, renal failure.

Introduction

The milk alkali syndrome also known as Burnett's Syndrome was first described by Hippocrates more than two thousand years ago [1]. The syndrome was commonly seen when milk and absorbable alkalis were the mainstays of treatment of symptoms due to acid related upper gastrointestinal disease [2]. Then, after the introduction of the non-absorbable alkalis and H2-receptor antagonists, the syndrome became rarer but did not disappear altogether. However, the milk alkali syndrome has always been suspected when patients present with hypercalcemia, renal impairment and metabolic alkalosis. The carbonate salts constitute the main stay of the milk alkali syndrome [3].

The modern version of milk alkali syndrome affects a different patient population and has a different etiologic basis than was characterized in the original descriptions of the syndrome [4]. Sodium and calcium carbonate salts are nowadays considered an environmental problem for the population, thus increasing the possibility of the appearance of more cases of the syndrome in the future. Natural sodium and calcium deposits are formed by a long geologic process of the erosion of igneous rocks. As they weather and break down, they are carried by water in rivers and streams thus undergoing collection and precipitation as carbonates in basins when in contact with carbon dioxide [5].

Deposits of sodium and calcium carbonates are found in large quantities in the United States, China, Botswana, Uganda, Kenya, Mexico, Peru, India, Egypt, South Africa and Turkey. The deposits are found both as extensive beds of sodium and calcium minerals called trona and nahcolite and as carbonate-rich waters (brines). The processing of minerals and brines for the production of soda ash is known as the alkali industry. While producing soda ash, a number of other sodium compounds are made as co-products, including sodium bicarbonate known as baking soda, sodium sulfite and the chemical caustic soda. The majority of soda ash is used to make glass and a variety of chemicals, followed by soaps and detergents, paper and paper pulp production, water treatment, in medicine, as a food additive, and other assorted uses. These other uses include oil refining, making synthetic rubber, and explosives [5].

At the workplace, inhalation of sodium and calcium carbonate dust may cause irritation of the respiratory tract followed by entry into the blood stream, resulting in elevation of measured bicarbonate levels [6].

Continuing exposure to carbonate salts and bicarbonate retention leads to alkalosis, which in turn causes increased calcium reabsorption in the distal collecting system of the kidney. The resulting hypercalcemia produces a renal concentrating defect that can be considered a form of nephrogenic diabetes insipidus [7].

Resultant dehydration and volume depletion may worsen the hypercalcemia which induces variable degrees of renal impairment and azotemia [8]. In milk alkali syndrome, when a large quantity of calcium is present in the blood, calcium deposition is not usual but hypercalcemia works to feedback on the parathyroid gland [8]. However, the cyclic pathophysiology of milk alkali syndrome that maintains hypercalcemia and alkalosis with continuous exposure is complex and not completely understood.

Accordingly, this work was carried out in order to study the effects of occupational exposure to limestone among workers in the alkali industry. Additionally, it is an attempt towards establishing the criteria for the diagnosis of chronic milk alkali syndrome. The importance of disease determination among exposed workers and among the general population was undoubtedly considered.

Aim of the Work:

This study was designed to investigate the effects of exposure to limestone and evaluate the undiagnosed cases with kidney affection and hypercalcemia detected among the involved workers. The study is also an attempt to highlight the importance and difficulty of diagnosing the milk alkali syndrome, being not an uncommon syndrome among the population.

Materials and Methods

Study Population:

This work was carried out in a petrochemical plant working in the production of soda ash from limestone cut from certain quarries near Alexandria. The studied population included 24 randomly selected individuals with exposure duration ranging from 14 to 38 years. An oral consent was obtained from the included subjects. Exclusion criteria included the intake of drugs such as calcium salts, hydralazine, lithium, thiazide diuretics and other medications that can increase the level of ionized calcium. The exposed population consisted of male subjects with age ranging from 38 to 59 years. The total amount of tobacco smoked per year also known as smoking index (SI) was chosen as a measure of current tobacco consumption [9]. As

for the control group, a total of 21 workers were chosen randomly from a group of school door keepers and bus drivers living and working in areas far from similar exposure. The control population matched the exposed groups in age, sex, social factors and special habits.

Clinical Assessment:

Detailed assessment questionnaire was used, interviewing the whole studied population which was then subjected to thorough clinical examination and electrocardiographic studies. Laboratory investigations were carried out to detect abnormalities in the levels of ionized calcium, phosphate, 1,25-dihydroxyvitamin D, parathyroid hormone (PTH) and parathormone hormone related peptides (PTH-rP). Arterial blood gas studies were done to estimate blood pH, oxygen and carbon dioxide tensions, bicarbonate, sodium, potassium and chloride as well.

Laboratory Investigations:

A small sample of 5ml venous blood was taken under complete aseptic conditions from all individuals. The sample was kept in a plain heparin-free tube, centrifuged and the amount of ionized calcium found in the serum was measured. The ionized calcium was assayed by ion-selective electrode using the Ciba-Corning 634 [10]. Serum inorganic phosphorus was measured by colorimetric method. Liver functions in the form of alanine transaminase (ALT) and aspartate transaminase (AST) and kidney functions (urea and creatinine), were estimated using Hitashi (911) autoanalyser. La Roche Germany supplied the kits and instruments.

1,25-Dihydroxyvitamin D (1,25-(OH)2-D) Analysis:

The vitamin D in plasma was measured by competitive protein- binding assay after isolation from plasma by single extraction and one-step chromatographic purification [11].

Parathyroid hormone/Parathormone Related Peptides (PTH/PTH-rP) Assays:

The intact PTH immunoassay is a two-site enzyme-linked immune-sorbent assay (ELISA) for the measurement of the biologically intact 84 amino acid chain of PTH using goat polyclonal antibodies. One antibody is prepared to bind only the mid-region and C-terminal PTH (amino acids 39-84) and this antibody is biotinylated. The other antibody is prepared to bind only the N-terminal PTH (amino acids 1-34) and this antibody is labeled with horseradish peroxidase for detection. The samples are incubated with the enzyme

labeled antibody and the biotin coupled antibody in a streptavidincoated microplate well. At the end of incubation, the micro-well is washed to remove unbound components and the enzyme bound to solid phase is incubated with the substrate tetramethylbenzidine. An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of intact PTH in the sample. Concentrations of intact PTH present are determined directly from a dose response curve of absorbance unit versus concentration [10]. Similarly, the serum PTH-rP was measured using a two-site immune-radiometric assay, as previously described, but using affinity-purified anti-PTH-rP (amino acids 37-74) as the capture antibody bound to a solid phase and radio-labeled anti-PTH-rP (amino acids 1-36) as the signal antibody.

Arterial Blood Gases (ABG) Analysis:

Prior to radial artery puncture, local anesthesia was applied to the skin or tissues surrounding the radial artery. An experienced technician collected an arterial sample of at least 4 ml using a 1-inch 22-gauge thin-wall needle attached to a five-ml glass syringe with 0.2 ml of liquid sodium heparin (1,000 U/ml) in the syringe dead space. All specimens were stored in ice-water slurry as the samples could not be analyzed immediately. Specimens were analyzed on automated blood gas analyzers using an electrode temperature of 37°C [12].

Electrocardiographic Analysis:

An electrocardiogram (ECG) was done for each subject of the studied population to record the electrical activity of the heart using an ECG machine. Small metal electrodes were stuck onto the arms, legs and chest. Wires from the electrodes were connected to the ECG machine. The machine detected and amplified the electrical impulses that occur with each heartbeat and recorded them onto a paper or computer. As QT interval, measured from the start of Q wave to the end of the T wave, is dependent on the heart rate in an obvious way (the faster the heart rate, the shorter the QT interval), the QT interval had to be adjusted to aid interpretation. The suggested approach used the formula [QTc = QT + 0.154 (1-RR)] where QTc is the corrected QT interval while RR is the interval from the onset of one QRS complex to the onset of next QRS complex measured in seconds [13].

Statistical Analysis:

The mean and standard deviation (SD) were calculated. Unpaired student's t-test

was used to evaluate the relation between the different indices and compare the two study groups. Pearson correlation coefficient was used to relate between the age, duration of exposure, smoking index, ionized calcium, phosphate, 1,25-dihydroxyvitamin D, parathyroid hormone, PTH-rP, sodium, potassium, chloride and the arterial blood gases parameters. A chi square test was performed for statistical analysis of qualitative parameters. A P value <0.05 was considered significant.

Results

This study was carried out on a group of workers exposed to limestone in a petrochemical plant near Alexandria working in the production of soda ash and a group of door keepers and bus drivers from a distant school considered as control. Both selected groups were matching for age, sex, social conditions and smoking habits. The mean ± SD of age was 51.33±5.12 years among the exposed group and 48.91±5.81 years among the control group. As regards the smoking index, the mean ±SD were 27.25±6.38 and 20.38±5.20 pack.year among the exposed group (n=24) and the control group (n=21) respectively. The duration of exposure for the exposed population ranged from 14 to 38 years with a mean of 22.79±7.28 years.

The studied population was questioned for full medical and occupational history and was subjected to clinical examination. Evaluation of obtained data, results not presented, revealed the presence of fatigue (20; 83.3%) and malaise (8; 33.3%), to a lesser extent lack of concentration and mental confusion (2; 8.3%). Among control group, the corresponding manifestations were present in (12; 57.1%), (2; 9.5%), (0%) and (1; 4.8%), respectively, with no significant statistical difference detected. The gastrointestinal, genitourinary and cardiovascular manifestations included presence of nausea (14; 58.3%); infrequent vomiting (7; 29.2%) and constipation (17; 70.8%) among exposed workers compared to (5; 23.8%); (0%) and (3; 14.3%) respectively, among the control group. Urinary frequency was complained of among 19 (79.2%) exposed workers compared to 6 (28.6%) control cases who were previously diagnosed as urinary tract infections and were under treatment.

As regards the cardiovascular manifestations and ECG changes, palpitation was complained of by 15 cases (62.5%) of the exposed population as evidenced by the presence of supra-ventricular (SV) extrasystoles and sinus tachycardia with a rate

of 96 to 142 beats per minute in the electrocardiogram (ECG). Evaluation of the ECG changes among the control group revealed sinus tachycardia among 9 cases (42.9%) whereas SV extrasystoles were detected among only 2 cases (9.5%). Short QT-interval was seen in 5 (20.8%) workers of the exposed population with no such changes among the control group. No statistically significant difference was detected between the two groups as regards the clinical data.

The liver functions, serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) enzymes were within normal limits among the studied population with no significant statistical difference between the groups. Evaluation of kidney functions revealed non-significant elevation of creatinine among the studied population in contrast to statistically significant elevation of urea among the exposed workers compared to the control group. Estimation of ionized calcium (ion-Ca), phosphorus (P), 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D), parathyroid hormone (PTH) and parathormone hormone related peptides (PTH-rP) revealed a statistically highly significant elevation of ionized Ca, significant elevation of PTH, but highly significant reduction of PTH-rP (P value <0.005) among exposed workers compared to control. As for phosphorus and 1,25-dihydroxyvitamin D, the reduction in exposed workers was non-significant statistically (Table 1).

Arterial blood gases analysis was performed for both exposed and control groups. The data obtained revealed insignificant difference between the results of all parameters except for the pH which showed a difference statistically highly significant between the groups. The mean values obtained were 7.46±0.03 among the exposed group and 7.39±0.03 among the control group. The anion gap that appears on laboratory testing because certain cations and anions are not measured on routine laboratory chemistry panels was subsequently calculated. The anion gap estimated among the exposed population was found to be 13.21±1.21 mEq/L. The elevation detected above the typical anion gap which equals 12 mEq/L was used to indicate metabolic acidosis, albeit non-significant. Accordingly, delta gap, which is the difference between calculated and normal anion gaps, was added to measured bicarbonate. The resulting HCO_3^- equivalent, which should be in normal range with measured HCO_3^- values, revealed significant statistical difference between exposed

(25.75±2.29 mEq/L) and control groups (20.38±1.66 mEq/L), therefore indicating the presence of metabolic alkalosis (Table 2). Winter's formula, comparing the predicted [1.5 (HCO_3^-) + 8±2] and measured PCO_2 reflected an appropriate respiratory compensation and no secondary acid-base disorder (data not shown).

Analyzing the effect of age, smoking index and duration of exposure on the different laboratory parameters was carried out among the studied population. A non-significant correlation was obtained for the effect of age versus all studied parameters. Analysis of the duration of exposure among the exposed population revealed a significant negative association with the level of ionized calcium and a positive association with the level of intact parathyroid hormone. As for the other laboratory parameters, the association was non-significant. The smoking index showed a positive correlation with kidney functions which proved to be highly significant for urea and significant for serum creatinine. Positive associations were obtained with the liver functions and ionized calcium, but were found to be highly significant for the intact PTH only. As for the phosphorus, 1,25-dihydroxyvitamin D and PTH-rP, the negative associations obtained were significant with phosphorus and highly significant with the others (Table 3).

The correlations of age, smoking index and duration of exposure with blood gases and with the sodium, potassium and chlorides were also evaluated. Age in years showed no significant influence on these parameters. As for smoking, a significant association was obtained with oxygen tension, carbon dioxide tension, bicarbonate equivalent, and the association was highly significant with pH (P value <0.005). Blood pH was found to be the only parameter associated significantly with the duration of exposure (Table 4).

Further relationships studied between the different variables in the study showed highly significant associations for urea with the levels of ionized calcium, phosphorus, 1,25-dihydroxyvitamin D, the intact PTH and PTH-rP. Similarly, the associations with creatinine were highly significant for the same parameters except for a non-significant association with phosphorus in serum. As regards liver enzymes, a significant inverse relationship was only obtained for the 1,25-dihydroxyvitamin D. Ionized calcium and the intact PTH proved to be positively related highly significantly. They both showed negative highly significant associ-

ations with the phosphorus, vitamin D and PTH-rP. Alternately, phosphorus was associated positively with the 1,25-dihydroxyvitamin D and PTH-rP. The PTH-rP and 1,25-dihydroxyvitamin D were found to positively correlate together highly significantly (Table 5).

Correlation of kidney functions with arterial blood gases showed high significant associations, positively with blood pH and measured bicarbonate, and negatively with oxygen tension. The additional positive associations of kidney functions with carbon dioxide tension, bicarbonate equivalent, anion gap, sodium and potassium proved to be significant with creatinine and highly significant with urea. However, no significant association was detected with the chloride. The associations of ionized calcium and parathyroid hormone were found directly related with pH, carbon dioxide tension, measured and equivalent bicarbonates, anion gap and sodium ions, while indirectly with the oxygen tension, potassium and chloride. Correlation of calcium proved highly significant with all parameters but non-significant with sodium; as for the parathyroid hormone, all associations were highly significant except for the chloride which was non-significant (Table 6).

On the other hand, the phosphorus, 1,25-dihydroxyvitamin D and PTH-rP were directly correlated with oxygen tension, potassium and chloride, while indirectly with pH, carbon dioxide tension, measured and equivalent bicarbonates, anion gap and sodium ions. The phosphorus was associated significantly with pH, sodium and potassium, highly significantly with oxygen and carbon dioxide tension, measured and equivalent bicarbonate and anion gap, however, non-significantly with chloride ions. The associations of 1,25-dihydroxyvitamin D and PTH-rP with pH, oxygen and carbon dioxide tension, measured and equivalent bicarbonates proved to be highly significant but was significant with sodium ions. Furthermore, the association with 1,25-dihydroxyvitamin D was significant with anion gap, highly significant with potassium but non-significant with chloride. As for the PTH-rP, the associations were found highly significant with the anion gap and significant with both potassium and chloride (Table 6).

Table (1): Mean ±SD of the results of Different Laboratory Investigations among the Group of Workers exposed to Soda Ash (n=24) and the Non-exposed Control Group (n=21).

		Exposed Group n = 24	Control Group n = 21	t-test	P value
Urea	Range Mean SD	30.2 - 50.6 38.500 5.051	19.8 - 34.2 22.567 3.608	2.4875	0.0168*
Creatinine	Range Mean SD	0.82 - 2.04 1.294 0.383	0.7 - 1.34 0.938 0.157	0.0003	N.S.
ALT	Range Mean SD	5.8 - 56.3 25.496 14.777	12.6 - 45.7 22.690 7.485	0.4365	N.S.
AST	Range Mean SD	14.1 - 56.2 34.546 13.318	13.8 - 46.2 28.771 9.503	0.1059	N.S.
P	Range Mean SD	1.04 - 5.2 3.274 1.160	3.7 - 5.75 4.649 0.640	1.7938	N.S.
Ion-Ca	Range Mean SD	7.2 - 9.5 8.318 0.717	4.7 - 6.6 5.169 0.667	7.0865	0.0001**
1, 25-$(OH)_2$-D	Range Mean SD	87.3 -121.4 103.042 10.442	122.9-134.8 129.771 3.051	1.8544	N.S.
PTH	Range Mean SD	38.6 - 92.6 59.904 15.532	29.4 - 42.9 35.724 4.586	1.9984	0.052*
PTH-rP	Range Mean SD	17.4 - 25.6 20.468 2.325	19.5 - 29.8 26.629 2.937	7.8352	0.0001**

Urea (mg/dl); Creatinine (mg/dl); ALT: Alanine Transaminase (IU/L); AST: Aspartate Transaminase (IU/L); P: Phosphorus (mg/ml); Ion-Ca: Ionized calcium (mg/ dl); 1,25-$(OH)_2$-D: 1,25-hydroxyvitamin D (ng/ml); PTH: intact Parathyroid hormone (pg/ml); PTH-rP: parathormone hormone related peptides (pg/ml).

NS: Non-significant P>0.05 * Significant P<0.05

**Highly Significant P<0.005.

Table (2): Mean ±SD of the Arterial Blood Gases Results as well as the Sodium (Na), Potassium (K) and Chloride (Cl) Ions among Workers exposed to Soda Ash (n=24) and the Non-exposed Control Group (n=21).

		Exposed Group n = 24	Control Group n = 21	t-test	P value
pH	Range Mean SD	7.4 - 7.51 7.464 0.030	7.35 - 7.42 7.387 0.028	3.4883	0.0011**
PO_2	Range Mean SD	85 - 94 89.792 2.889	91 - 97 93.429 1.859	1.2344	N.S.
PCO_2	Range Mean SD	41 - 56 49.625 5.686	34 - 41 37.667 2.058	1.3155	N.S.
Na^+	Range Mean SD	132 - 147 138.375 4.126	132 - 142 135.429 2.336	0.0061	N.S.
K^+	Range Mean SD	3.2 - 4.2 3.592 0.341	3.6 - 4.5 4.052 0.269	1.0999	N.S.
Cl^-	Range Mean SD	95 - 109 102.625 3.910	101 - 110 104.952 2.747	0.0277	N.S.
$mHCO_3^-$	Range Mean SD	18 - 25 22.542 1.641	17 - 22 19.143 1.590	1.1593	N.S.
Anion gap	Range Mean SD	10 - 17 13.208 2.207	10 - 13 11.333 0.913	0.0008	N.S.
$eHCO_3^-$	Range Mean SD	22 - 31 25.75 2.289	17 - 23 20.381 1.658	2.6606	0.0109*

PO_2: Oxygen tension (mmHg): PCO_2: Carbon dioxide tension (mmHg);
Na: Sodium (mEq/L); K: Potassium (mEq/L); Cl: Chloride (mEq/L);
$mHCO_3^-$: Bicarbonate Measured (mEq/L); $eHCO_3^-$: Bicarbonate Equivalent (mEq/L); Anion gap (mEq/L).
NS: Non-significant P>0.05
* Significant P<0.05
**Highly Significant P<0.005

Table (3): Correlation between the Kidney and Liver Functions as well as the Serum Levels of Ionized Calcium, Phosphorus, 1,25-Dihydroxyvitamin D, Parathyroid hormone and Parathormone Hormone Related Peptides versus the Age, Smoking Index (n=45) and the Duration of Exposure (n=24).

		Age	Exposure Duration	Smoking Index
Urea	r value	0.2735	0.2556	0.4743
	p value	0.069	0.228	0.001**
Creat	r value	01226	0.0079	0.3537
	p value	0.422	0.971	0.017*
ALT	r value	-0.1082	0.1296	-0.1081
	p value	0.479	0.546	0.479
AST	r value	0.1336	-0.1252	0.0412
	p value	0.382	0.559	0.788
Ion-Ca	r value	0.2232	-0.4118	0.0438
	p value	0.141	0.046*	0.775
P	r value	-0.0639	-0.2808	-0.3373
	p value	0.677	0.062	0.024*
1, 25-(OH)2-D	r value	-0.1517	-0.092	-0.4761
	p value	0.320	0.670	0.000**
PTH	r value	0.0682	0.2949	0.4533
	p value	0.656	0.049*	0.002**
PTH-rP	r value	-0.0966	-0.3313	-0.5362
	p value	0.528	0.114	0.000**

Age (years); Exp: Exposure Duration (years); SI: Smoking Index (pack.year);

Urea (mg/dl); Creat: creatinine (mg/dl);

ALT: Alanine Transaminase (IU/L); AST: Aspartate Transaminase (IU/L);

Ion-Ca: Ionized calcium (mg/dl); P: Phosphorus (mg/ml); 1,25-(OH)2-D: 1,25-hydroxyvitamin D (ng/ml);

PTH: Intact parathyroid hormone (pg/ml); PTH-rP: parathormone hormone related peptides (pg/ml).

NS: Non-significant P>0.05 * Significant P<0.05 **Highly Significant P<0.005.

Table (4): Correlation between the Arterial Blood Gases, Sodium, Potassium, Chloride, measured and equivalent Bicarbonate and the Anion Gap versus the Age, Smoking Index (n=45) and Exposure Duration (n=24).

		Age	Exposure Duration	Smoking Index
pH	r value	0.0922	0.5140	0.4614
	p value	0.547	0.01*	0.001**
PO_2	r value	-0.0612	-0.1449	-0.3008
	p value	0.689	0.499	0.045*
PCO_2	r value	0.0678	-0.1228	0.3531
	p value	0.658	0.568	0.017*
$mHCO_3^-$	r value	-0.0221	0.0681	0.2625
	p value	0.886	0.752	0.082
$eHCO_3^-$	r value	-0.0369	0.2369	0.3288
	p value	0.809	0.265	0.027*
Anion	r value	-0.0084	0.1951	0.2883
	p value	0.956	0.361	0.05*
Na	r value	-0.0847	0.2503	-0.0861
	p value	0.580	0.238	0.574
K	r value	-0.0209	0.1183	-0.2251
	p value	0.891	0.582	0.137
Cl	r value	-0.0682	0.1255	-0.2408
	p value	0.656	0.559	0.111

Age (years); Exp: Exposure Duration (years); SI: Smoking Index (pack.year);

PO_2: Oxygen tension (mmHg): PCO_2: Carbon dioxide tension (mmHg);

Na: Sodium (mEq/L); K: Potassium (mEq/L); Cl: Chloride (mEq/L);

$mHCO_3^-$: Bicarbonate Measured (mEq/L); $eHCO_3^-$: Bicarbonate Equivalent (mEq/L);

Anion gap (mEq/L).

NS: Non-significant P>0.05

* Significant P<0.05 **Highly Significant P<0.005

Table (5): Correlation between the Serum Levels of Ionized Calcium, Phosphorus, 1,25-Dihydroxyvitamin D, Parathyroid hormone and Parathormone Hormone Related Peptides as well as versus the Levels of Urea, Creatinine and Liver Functions among the Studied Population (n=45).

		Ion-Ca	P	1,25-D	PTH	PTH-rP
Urea	r value	0.7658	-0.4574	-0.7626	0.5335	-0.7051
	p value	0.000**	0.002**	0.000**	0.000**	0.000**
Creat	r value	0.4952	-0.1142	-0.4785	0.4477	-0.4880
	p value	0.000**	0.455	0.000**	0.002**	0.000**
ALT	r value	0.0289	-0.0580	-0.1012	0.2397	-0.1594
	p value	0.850	0.705	0.508*	0.113	0.296
AST	r value	-0.1013	-0.2118	-0.3116	0.2570	-0.3194
	p value	0.508	0.163	0.037*	0.088	0.319
Ion-Ca	r value	---	-0.4657	-0.7509	0.5543	-0.6533
	p value	---	0.001**	0.000**	0.000**	0.000**
P	r value	-0.4657	---	0.5194	-0.6582	0.5362
	p value	0.001**	---	0.000**	0.000**	0.000**
1,25-D	r value	-0.7509	0.5194	---	-0.7127	0.6609
	p value	0.000**	0.000**	---	0.000**	0.000**
PTH	r value	0.5543	-0.6582	-0.7127	---	-0.6797
	p value	0.000**	0.000**	0.000**	---	0.000**
PTH-rP	r value	-0.6533	0.5362	0.6609	-0.6797	---
	p value	0.000**	0.000**	0.000**	0.000**	---

Urea (mg/dl); Creat: creatinine (mg/dl);
ALT: Alanine Transaminase (IU/L); AST: Aspartate Transaminase (IU/L);
Ion-Ca: Ionized calcium (mg/dl); P: Phosphorus (mg/ml); 1,25-D: 1,25-hydroxyvitamin D (ng/ml);
PTH: Intact parathyroid hormone (pg/ml); PTH-rP: parathormone hormone related peptides (pg/ml).
NS: Non-significant P>0.05
* Significant P<0.05 **Highly Significant P<0.005

Table (6): Correlation between the Kidney Functions and the Serum Levels of Ionized Calcium, Phosphorus, 1,25-Dihydroxyvitamin D, Parathyroid Hormone and Parathormone Hormone Related Peptides versus Blood Gases, Sodium, Potassium, Chloride, measured and equivalent Bicarbonate and the Anion Gap (n=45).

		Urea	Creat	Ion-Ca	P	1,25-D	PTH	PTH-rP
pH	r value	0.7934	0.4018	0.7059	-0.3626	-0.6716	0.5635	-0.6829
	p value	0.000**	0.006**	0.000**	0.014*	0.000**	0.000**	0.000**
PO_2	r value	-0.4576	-0.4806	-0.5432	0.4782	0.5571	-0.4508	0.4884
	p value	0.002**	0.000**	0.000**	0.001**	0.000**	0.002**	0.000**
PCO_2	r value	0.7018	0.3118	0.7859	-0.5503	-0.7486	0.5097	-0.5688
	p value	0.000**	0.037*	0.000**	0.000**	0.000**	0.000**	0.000**
$mHCO_3^-$	r value	0.6288	0.5673	0.6639	-0.4690	-0.7135	0.5786	-0.4988
	p value	0.000**	0.000**	0.000**	0.001**	0.000**	0.000**	0.000**
$eHCO_3^-$	r value	0.6926	0.3825	0.7178	-0.5792	-0.7282	0.6677	-0.6590
	p value	0.000**	0.01*	0.000**	0.000**	0.000**	0.000**	0.000**
Anion	r value	0.4143	0.3071	0.4228	-0.4071	-0.3715	0.4397	-0.5453
	p value	0.005**	0.04*	0.004**	0.006**	0.01*	0.003**	0.000**
Na	r value	0.9646	0.2961	0.2434	-0.3627	-0.3766	0.4658	-0.3171
	p value	0.000**	0.05*	0.107	0.014*	0.01*	0.001**	0.034*
K	r value	0.4413	0.3171	0.5529	-0.3498	-0.5903	0.4972	-0.3830
	p value	0.002**	0.034*	0.000**	0.019*	0.000**	0.000**	0.01*
Cl	r value	0.1220	-0.0485	-0.4152	0.1560	0.2824	-0.1394	0.2980
	p value	0.425	0.752	0.005**	0.306	0.06	0.361	0.05*

Urea (mg/dl); Creat: creatinine (mg/dl);
Ion-Ca: Ionized calcium (mg/dl); P: Phosphorus (mg/ml); 1,25-D: 1,25-hydroxyvitamin D (ng/ml);
PTH: intact parathyroid hormone (pg/ml); PTH-rP: parathormone hormone related peptides (pg/ml);
PO_2: Oxygen tension (mmHg): PCO_2: Carbon dioxide tension (mmHg);
Na: Sodium (mEq/L); K: Potassium (mEq/L); Cl: Chloride (mEq/L);
$mHCO_3^-$: Bicarbonate Measured (mEq/L); $eHCO_3^-$: Bicarbonate Equivalent (mEq/L); Anion gap (mEq/L).
NS: Non-significant P>0.05 * Significant P<0.05 **Highly Significant P<0.005

Discussion

For ages, soda ash used to be produced synthetically by the Solvay process using salt, ammonia and limestone. The limestone was found to be composed of 40-100% by weight calcium carbonate ($CaCO_3$) in addition to the oxides of silicon, aluminum, iron, magnesium, calcium, sodium and potassium. However, the waste products of this process were harmful to the environment and could cause serious waste management problems [14]. The Solvay process was the main source of soda ash until the Wyoming trona deposits were discovered. Recently, soda ash is also obtained both by the processing of the minerals trona and nahcolite, and by the processing of brines which are found in large quantities in Egypt [5].

Soda ash has been produced for a long time, but neither accidental exposures nor chronic manifestations have been reported in the medical literature. In the current study, no environmental assessment was carried out at the workplace, but previous assessment of occupational exposure reported levels of silicon oxide less than the OSHA permissible respirable levels of 10 mg/m^3 compared to highly elevated calcium carbonate levels exceeding the 15 mg/m^3 OSHA-PEL for total dust [14]. Prolonged renal complaints were mentioned among workers, but to our knowledge, these symptoms have not been well investigated thus far. This work was therefore carried out to study the relationship of kidney complaints to limestone exposure. It also attempts to determine if the undiagnosed disease is a chronic form of the milk alkali syndrome which was formerly recognized as a disorder among patients taking calcium and alkali for the treatment of upper gastrointestinal hyperacidity.

Variable non-specific general and gastrointestinal disturbances as well as urinary frequency were detected among workers exposed to limestone in contrast to the control group with a statistically non-significant difference. Similarly, no statistical difference was obtained as regards the cardiac symptoms which were diagnosed electrocardiographically in the form of arrhythmias and shortened QT interval. Cardiac dysfunction with reduced ejection fraction has been related, in many studies, to kidney disease [15]. In chronic kidney disease, mineral and hormonal abnormality was observed at an early stage, leading to a systemic mineral calcium elevation with consequent extremely high cardiovascular morbidity and mortality [16].

In the current study, elevation of estimated ionized calcium, that is physiologically active and under homeostatic control, was found in the range from 7.2 to 9.5 mg/dl among limestone exposed workers with a mean value of 8.32±0.72mg/dl. Earlier studies revealed that patients with hypercalcemia may be completely asymptomatic whereas a wide variation of symptoms usually occurs, only if the serum calcium is above 12 mg/dl [17]. Additionally, no severe mental changes such as obtundation or coma were reported among exposed workers as such symptoms as well do not develop except when the serum calcium levels rise to higher than 15 mg/dl, being related to kidney complications [18].

The chronic kidney disease initially is without specific symptoms and can only be detected as an increase in serum creatinine [19]. In the current study, estimation of blood levels of creatinine among exposed group showed elevation with a mean value of 1.29±0.38 mg/dl. As for urea, significant elevation compared to control group was revealed (38.5±5.05 mg/dl) correlating positively and highly significantly with sodium and potassium levels. Few studies reported that further decrease in kidney functions is always characterized by accumulation of urea in association with increased potassium levels ending in symptoms ranging from malaise to cardiac arrhythmias [19].

As renal functions decline in chronic kidney disease, fibroblast growth factor-23 (FGF-23), that is a circulating factor regulating phosphorus and vitamin D homeostasis, increases. The FGF-23 has a physiological role in preventing tissue damage by ectopic calcification, partly via suppressing the serum calcium x phosphate product by accelerating the urinary phosphate excretion and partly by suppressing vitamin D activation [20]. As a fact, current evaluation of exposed workers showed low levels of phosphorus (3.27±1.16 mg/dl) and of active vitamin D (103.04±10.44ng/ml) to below normal values.

The steroid hormone 1,25-$(OH)_2$-D_3 is the major biologically active metabolite of the vitamin D sterol family. The vitamin D precursor undergoes hydroxylation in the liver at the C-25 position to form 25-hydroxyvitamin D, which is further hydroxylated at the C-1 position in the proximal nephron of the kidney [21]. Liver functions estimated among the exposed population proved to be within normal limits indicating proper 25-hydroxylation process in the liver and normal rate of pro-

tein synthesis. The vitamin D binding protein produced by the liver plays an additional role in maintaining stable serum stores of vitamin D metabolites and in modulating the rates of its bioavailability, activation and end-organ responsiveness necessary to maintain normal serum calcium homeostasis [22].

Yet, the renal hydroxylation is the major recognized control point in vitamin D metabolism, responding to ambient phosphorus and calcium concentrations and to circulating parathyroid hormone (PTH) concentrations. The PTH and the phosphorus depletion act independently to increase 1,25-$(OH)_2$-D_3 production, PTH level being the more potent stimulus. With vitamin D deficiency, the parathyroid hormone is increased to supply calcium through mobilization from bones [23]. The intact PTH estimated in the present study was found to be significantly higher among exposed workers showing a highly significant negative correlation with 1,25-dihydroxyvitamin D (P value < 0.005). Recently, a negative correlation was reported between the PTH and serum 25-hydroxyvitamin D, though non-significantly [24].

The three primary areas that respond to parathyroid hormone are all bone surface areas in contact with extracellular fluid, kidneys and indirectly the intestinal tract [25]. However, some tumor peptides PTH-related protein (PTH-rP), discovered in tumors derived from the kidney and other organs, were found to mimic the biological effects of PTH on bone, kidneys and the gut. The tumor peptides were found to share considerable homology with PTH in the first 13 amino acids, therefore binding to and activating the PTH receptors [21]. Estimation of the parathormone hormone related peptides (PTH-rP), therefore carried out, revealed significant lower levels among the exposed group compared to the control group. The presence of low levels of PTH-rP was considered evidence towards the exclusion of any controversial non-bone effects of PTH. Yet, the physiological role of the PTH-rP remains unclear with probably no regulatory effect on calcium homeostasis under physiological conditions as the PTH receptors are not as responsive to PTH-rP as they are to PTH [21].

The intact PTH therefore regulates renal calcium and phosphorus transport, the latter action being important to support the overall homeostatic role of PTH. The phosphorus is not needed typically, since dietary lack is infrequent, so PTH promotes phosphorus excretion by blocking

its re-absorption [26]. Simultaneously, PTH works at distal tubular sites in the kidney to lower the amount of urinary calcium excreted and increase renal calcium re-absorption [27]. An explanation might therefore be provided for the negative correlation of PTH with reduced phosphorus ($r = -0.6582$), and the positive correlation with increased calcium ($r = 0.5543$), both associations appeared highly significant.

Most studies of parathyroid hormone and calcium have focused on the modification of parathyroid hormone secretion by serum calcium, but the relationship between parathyroid hormone and serum calcium is bi-functional because the parathyroid hormone also modifies serum calcium [25]. The primary rapid action of the hormone is to set and maintain the free calcium concentration of the extracellular fluid which requires continuous secretion of parathyroid hormone [28].

However, elevation of blood levels of calcium exerts some kidney changes lowering the glomerular filtration rate directly by inducing renal vasoconstriction and indirectly by reducing extracellular volume. In the collecting tubules, the stimulated calcium-sensitive receptors cause an isotonic polyuria by blocking the action of antidiuretic hormone [3]. As a fact, increased urinary frequency was reported to be one of the common symptoms detected among the exposed workers in the present study. As for the renal tubules, the developing hypercalcemia increases the bicarbonate absorption ending in metabolic alkalosis. However, at room temperature, carbonate ions in the workplace react with the mucus film lining the respiratory tract, with the formation of bicarbonate and hydroxide [14], causing aggravation of the metabolic alkalosis.

Analysis of arterial blood gases among exposed workers showed elevation of blood pH indicating the presence of alkalemia with a mean value of 7.46 ±0.03. Estimation of the bicarbonate serum levels indicated elevation of measured bicarbonate (22.54±1.64mEq/L), though within normal limits. The anion gap was then calculated to determine if changes in HCO_3^- are primary or compensatory [29]. A highly significant difference in the anion gap between both exposed and control groups was detected, the mean values were (13.21 ± 2.21 and (11.33±0.91)mEq/L, respectively. Increase in the anion gap above the typical 12 mEq/L reflected metabolic acidosis due to increased anions most commonly caused by uremia as evidenced by a highly significant positive association for

urea versus the anion gap and measured bicarbonate.

As metabolic acidosis was present, the delta gap which is the difference between the patient's anion gap and the normal anion gap was calculated. By addition of the delta gap to the measured HCO_3^-, the result which should be in the normal range for HCO_3^- was considered. However, the HCO_3^- equivalent calculated was 25.75 ± 2.29 mEq/L. The metabolic alkalosis was therefore suggested by elevation of HCO_3^- above 28 mEq/L. Estimation of the PCO_2 revealed an elevation in the range from 41 up to 56 mmHg, indicating the presence of respiratory compensation and the absence of secondary acid-base disorder when applying Winter's formula [30]. In the present study, a highly significant positive correlation was obtained between blood pH and the bicarbonate equivalent versus the intact PTH and ionized calcium. Previous studies considered metabolic alkalosis an important factor in reducing the stimulation of the calcium-sensitive receptors on the parathyroid chief cells contributing to the increased production of parathyroid hormone and stimulation of PTH receptors with subsequent hypercalcemia [31].

According to the previous results, the diagnosis of the milk alkali syndrome can be achieved depending on the history of exposure to excess carbonates, the finding of hypercalcemia, renal impairment and metabolic alkalosis, and exclusion of other causes [3]. Earlier studies reported that the pathophysiology of the syndrome is complex. While the increased exposure to calcium and sodium carbonates must play a part, the central abnormality is a reduction in the ability of the kidneys to excrete calcium. As part of this reduction is due to the hypercalcemia itself the possibility exists that a vicious circle will develop. The reduced excretion of calcium results, not only from reduction in glomerular filtration rate but also, from increase in tubular reabsorption of calcium due to alkalosis [31]. Milk-alkali was considered the third leading cause of hypercalcemia of any degree and the second cause of severe hypercalcemia among individuals without end stage renal disease [18].

The extent and reversibility of the renal failure depend on the duration and severity of the milk alkali syndrome [3]. A significant association was found between the levels of ionized calcium and PTH versus the duration of exposure at the alkali industry in the current study, and accordingly versus the increased blood alkalemia as evidenced by the significant positive

correlation. Age might not be considered a confounding factor as no significant association was detected with the different parameters under study. A recent research proved the serum PTH not to vary with age compared to the serum 25-hydroxyvitamin D which was found to decrease with age [24].

As for the smoking index, few researches have excluded tobacco smoke as a factor in the causation of chronic renal failure and in the causation of calcium and parathyroid hormone disturbances as no differences in total serum calcium or phosphorus between smokers and nonsmokers were detected. The results reflected relative insensitivity of these parameters to changes in tobacco exposure, very likely because of their efficient regulating mechanisms [32]. However, in this study, the estimated smoking index showed highly significant correlations, negatively with the 1,25-$(OH)_2$-D and positively with the PTH. These current data might be supported by other previous studies showing a wide range of serum calcium levels in smokers indicating the increased tobacco-induced bone resorption by alterations in the vitamin D-PTH axis [33], and also importantly by influencing the calcium-regulating hormone [34]. These results supported the earlier data documenting the association between smoking and vitamin D metabolism derangement [35].

Associations of smoking index values with blood gases revealed a significant negative correlation with oxygen tension and a positive correlation with carbon dioxide tension and blood pH, anion gap as well as the equivalent bicarbonates. The level of PaO_2 was found to be strongly disturbed in smokers at rest as well as during moderate exercise, despite the lack of correlation between PaO_2 and the intensity of tobacco consumption expressed as number of pack-years [32]. As regards the current results, smoking might be excluded as a confounding factor as the statistical difference between the exposed and control groups was found to be non-significant.

Therefore, the milk alkali syndrome which was formerly recognized as a medical disease among individuals with peptic ulcer disease nowadays seems to experience resurgence in its incidence largely due to wider occupational exposure during the processing of soda ash. The modern version of the chronic form of milk alkali disease is therefore affecting a different type of the population occupationally exposed and is depending on a different etiologic basis than was characterized in the

original descriptions of the syndrome.

Unfortunately, the relatively small size of the data set in this study might be considered a limitation of the work, thus more wide scale researches are required to examine the complex interplay between the exposure to sodium and calcium carbonates and kidney activity for future protection as progress into renal failure is inevitable. Milk-alkali syndrome is by no means a new pathologic entity but is one that has re-emerged with a frequency that had only been seen in its remote historical past. The factors accounting for the increase, however, are different and range across a much wider medical spectrum than the former association with the treatment for peptic ulcer disease. Currently, calcium in the form of calcium carbonate rather than milk products is the primary causative factor.

Therefore, the central point in diagnosing the disorder is the history of excess calcium carbonate exposure, which can be easily overlooked without a high index of suspicion. Additionally, full details of all medication should be obtained as most individuals are often unaware that many medications contain calcium and alkali. Once hypercalcemia is suspected, removal from exposure should be considered therefore the pathophysiologic stimulus for hypercalcemia will no longer be present. Hypercalcemia in this setting will probably be rapidly corrected. Yet, the primary therapy of hypercalcemia in milk-alkali syndrome is intravenous volume replacement with isotonic sodium chloride solution. Proper interference and management are therefore indicated by immediate admission to the hospital.

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