THE STUDY OF PATHOLOGICAL BIOMINERALS IN HUMANS: COMPOSITIONAL FEATURES AND ENVIRONMENTAL INFLUENCE ON BASILICATA (SOUTHERN ITALY) CASE STUDY

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OBJECTIVE

Analyze the geo-environmental factors, which can influence the kidney stones formation and identify the main types of kidney stones to be found in Basilicata, through a chemical-mineralogical and petro-morphologic study.

INTRODUCTION

Nephrolithiasis is a very spread disease affecting 1 to 15% of the world population (Ramello *et al.*, 2000; Schneider, 1985; Scott, 1985).

The prevalence of nephrolithiasis is different in the world: in Asia it is equal to 1-5%, in Europe it is equal to 5-9%, in North America it is equal to 13-15% and in Saudi Arabian it is equal to 18-20% (Hess, 2003; Lee, 2002; Serio & Fraioli, 1999). In Italy in the last ten years this pathology has increased significantly especially in Southern Italy (Baggio, 1999; Serio & Fraioli, 1999).

The first study on the composition of a kidney stone was carried out in 1800 by Schellee (1742-1786) and Bergman (1734-1794), who identified a stone of uric acid.

Until 20 years ago there was little medical knowledge of the causes and the treatment to be applied in nephrolithiasis and many therapies were based on empirical remedies.

In the last few years the scientific progress has made it possible to understand some environmental, physico-chemical and metabolic aspects related to the origin and the formation of kidney stones.

Nowadays this pathology still shows a high percentage of recurrences (75% after 10 years; Uribarri *et al.*, 1989) because in most of the cases no effective therapeutic treatment suitable for the kind of stone under study is applied.

It is also worth noticing that due to the multi-factorial aspect of this pathology a thorough knowledge requires a multidisciplinary approach including mineralogy, crystal-chemistry, kidney physiology, biochemistry and analytical chemistry.

In fact, the information on the composition of biominerals, that each patient produces, is important because it allows a classification of the patient according to a specific pathology so as to identify a suitable therapeutic treatment by modifying factors such as *p*H, calciuria, uricosuria, crystal inhibitors, etc. or identify an effective phyto-therapeutic treatment also considering the different reaction to the treatment through extracorporeal shock wave lithotripsy (ESWL) of the different mineralogical phases.

In the last ten years in Italy nephrolithiasis has significantly increased in Southern Italy and the Basilicata region. From 2005 to 2008 the hospitalizations for nephrolithiasis cost € 3,529,500 [http://www.sanita.basilicata.it] to the Regional Authority.

In the light of this, it is important to investigate the mineralogy, crystallography, texture, morphology and chemistry of kidney stones and urinary crystals produced by patients hospitalized in Basilicata hospitals as well as gather information on geo-environmental and behavioural factors affecting the development of nephrolithiasis in Basilicata.

Preliminary data (Baggio *et al.*, 2008; Giannossi & Summa, 2010; Giannossi *et al.*, 2008, 2009) demonstrate that the methodological approach suggested is found to be of interest for a better understanding of the pathology both from a treatment and prevention point of view.

MATERIALS AND METHODS

A three-year-long epidemiological study was carried out in order to identify the prevalent nephrolithiasis. A representative sample of the Basilicata inhabitants hospitalized at San Carlo Regional Health Service Trust in Potenza was chosen in order to find out their dietary and behavioural habits through the submission of a questionnaire during the observation period. The values of prevalence and/or incidence calculated were presented through graphs showing the number of new cases of nephrolithiasis in the function of time (epidemic curves) in order to highlight the tendency of the disease with respect to geographical distribution, too (prevalence maps).

In order to identify a statistical relation between a supposed factor (or exposition to a certain factor) and the development of kidney stones in Basilicata, parameters of statistical association will be determined: Odds ratio.

A morphological and compositional characterization together with the use of integrated techniques – optical and scanning electron microscopy and X-ray powder diffraction – were performed on more than 80 kidney stones collected in three years.

Some thin petrographic sections were obtained for a representative number of bigger kidney stones.

The amount of some chemical elements specifically involved in the kidney stone crystallization process (Ca, Mg) or potentially toxic (Pb, Cr) was found by means of optical and atomic absorption spectrometry

RESULTS AND DISCUSSION

The geographic epidemiological survey showed the regional nephrolithiasis distribution (6.49‰ on average) and the areas particularly at risk due to demographic, environmental and behavioural factors.

The prevalence is higher among men (7.12‰) than women (5.89‰), and increases with age. The risk is higher for men between 40 and 59.

In detail the prevalence rate sharply increases when compared with the regional average, and reaches values above 11‰ for the Potenza Health Authority (ASL n. 2).

The municipalities with a prevalence of kidney stones are distributed in the central north-western region (Apennine area).

Low temperatures, high altitude, low solar radiation and some soil characteristics are some of the risk factors which could explain the prevalence found, all of them influencing the Basilicata inhabitants' lifestyle.

A positive correlation between the development of kidney stones and the consumption of hard water (> 22.5°F) is also found.

A total of 80 kidney stones of different compositions were collected, photographed, observed under an optical and scanning electron microscope and analysed by X-ray powder diffraction.

The results show that 65% of the kidney stones studied are composed of calcium oxalates (38% calcium oxalate monohydrate – whewellite; 27% calcium oxalate dihydratate – weddellite). Trace quantities of other organic and inorganic components are still present. Calcium oxalates are absent or negligible in the remaining 35%, these being composed mainly of uric acid (15%), hydroxylapatite (13%), struvite (6%) or cystine (1%). 32% of stones are multi-composed (*e.g.* weddellite plus hydroxylapatite or whewellite plus uric acid).

The internal structure and the relations between the major and minor components provide information to classify the 80 kidney stones in 8 distinctive types and 13 subtypes in accordance with the scheme proposed by Grases *et al.* (1998), but improved on a new type of kidney stone made of weddellite mixed-struvite.

Among stones with predominant calcium oxalate, there is also a high percentage of papillary kidney stones consisting of whewellite developed on kidney wall. The core of this stone type can form exclusively on sites with altered (damaged or just slightly injured) epithelium (Giannossi *et al.*, 2009). Cells of damaged epithelium tend to accumulate calcium thus creating favourable conditions for the formation of kidney stones containing crystals which, under urinary conditions suitable for the crystallization, produce an important deposit of material on the damaged epithelium which serves as a substrate for ulterior nucleation of whewellite stones.

The results of the study on some thin petrographic sections, obtained for a representative number of bigger kidney stones, show that there are usually more cores within whewellite kidney stones which are the result of several simultaneous nucleation and growth processes. Cores appear as spherulitic aggregates, the form which best adapt to non-equilibrium conditions. Simultaneous crystallization and oscillation between growth and dissolution events contribute to stone formation.

A comparison between the regional and international prevalence rates (in the absence of national data) leads to interesting observations:

- the prevalent kidney stones appear to be those composed of calcium oxalate;

- in the Basilicata community there is a larger number of uric acid kidney stones (18%) and a lower quantity of those composed of calcium phosphate, that may be related to some specific risk factors mainly referable to dietary habits such as an excessive consumption of proteins and a consumption of soft water with a low bicarbonate content which does not facilitate urine alkalinisation.

This could justify the geographical distribution of the uric acid kidney stones found exclusively in the northern region, an area with a predominant soft water and characterized by a low solar radiation and average temperatures lower than the rest of the region. All these factors do not facilitate either fluid intake or a higher calcium absorption.

Trace elements have been found in kidney stones. Their role in lithogenesis is debated. They may be involved in crystal induction depending on the particular relations between metals and solutes able to crystallize in urine. It is important to highlight that kidney stones containing trace elements could be considered as environmental pollution markers.

48 kidney stones composed of calcium oxalate, calcium phosphates, uric acid, cystine and a mixture of these were analyzed for the content of specific chemical elements either involved in the kidney stone crystallization process (Ca, Mg, K, Zn, Fe, Cu, Mn) or potentially toxic (Pb, Cr).

Four main findings emerge from the results:

1) Most kidney stones collected have high concentrations of elements such as K, Cu and Mg and a low content of Fe when compared to the results obtained from other researches.

2) Appreciable amounts are found in inorganic phases (calcium oxalate e phosphates), whereas only Zn content is higher in organic phases (uric acid and cystine).

3) Among calcium-containing stones (more abundant), the calcium-phosphate ones contain greater amounts of trace elements than the calcium-oxalate ones, and among the calcium-oxalate ones weddellite retains more trace elements than whewellite. The former is the first hydrates phase which forms during the crystallization process and then becomes whewellite. During this process the trace elements released from weddellite are not considered in the new structures.

4) Toxic elements such as lead and chromium (80% of the amount absorbed is excreted in urine) may be triggers of disease affecting renal papilla, and the slightly higher content of the two elements in papillary whewellite kidney stones needs consideration.

Furthermore, the results show that the concentration of Zn, Cu, Fe, Pb and Cr is greater than that of a standard diet ingestion, therefore varying amounts of these elements may be attributed to their enrichment in the diet of the inhabitants of polluted areas. This issue will require in-depth experiments in vitro.

CONCLUSIONS

This scientific activity is a first example of Italian study of kidney stones carried out at regional level with a multidisciplinary approach which made it possible significant achievements in the field of human health protection.

The chemical-mineralogical and petro-morphological analysis, performed with integrated techniques on a large number of kidney stones, allowed the gathering of information useful for identifying the prevalent stone types and defining some geo-environmental risk factors.

In addition, a petrographic and mineralogical investigation into stones made the nucleation and growth processes more clear, especially for calcium oxalate kidney stones.

This morpho-compositional data are useful for classifying each type of kidney stone, and, therefore, each patient in more than 30 different subgroups characterized by specific etiologic factors necessary to determine the treatment and disease prevention, especially in the presence of mixed stones requiring proper intervention for each mineral phase present.

Kidney stones, being so widely spread in many stone types, can be considered as markers to evaluate the presence of trace elements, especially those potentially toxic, in human body as well as in the environment.

Several kinds of kidney stones with a new mineral assemblage have been found and this represents a further step forward in understanding this widespread disease and stimulates further research.

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