

## INVESTIGATION INTO FAMILIAL RELATIONSHIP IN PROTEIN PROFILE IN KIDNEY STONE DISEASE

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**Abstract:** Clinical facility for present study was available at Al-Bashir Hospital Sadiqabad. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970) was employed for studying the low molecular weight serum protein fractions of affected and control families. Gel photography and quantification of various protein fractions were carried out by Gene Genius Bio-imaging Gel Documentation system that provided the data of molecular weights and percent area covered by each of the fractions. Data was employed in finding the enhancement or reduction and appearance or disappearance of particular protein fraction for comparison between affected and control families. Six protein fractions were detected in control families. Protein fraction of 11kDa was detected only in affected families and could not be expressed in controls. Protein fractions of 17kDa and 20kDa exhibited significant decreases in affected families when compared to control families. Protein fraction of 14kDa that appeared in control was absent in most of the affected families.

**Key words:** Gel electrophoresis, nephrolithiasis, protein fractions

### INTRODUCTION

Nephrolithiasis or kidney stones are small hard crystals formed when substances like calcium, uric acid or magnesium ammonium phosphate precipitate out of urine and build up on inner surface of kidney (Good enough *et al.*, 1990). Stones are generally composed of xanthine. Among these most prevalent (75%) are calcium stones (Parums *et al.*, 1996). Stones develop when there are imbalances of components in urine that are due to one or a combinations of the following conditions; hypercalciuria, hyperuricosuria, hypocitraturia, and several other factors including environmental specifically the acute shortage of water, as in desert and arid areas, dietary habits, life style and genetic factors (Gault *et al.*, 1993 and Baggio *et al.*, 1999). The strong evidence of the importance of environmental factors in renal stone disease seems to diminish the role of genetic factors but these are clearly involved. According to some experts if there is too much Cl<sup>-</sup> in urine then there will be excessive amount of calcium to balance it. By using new technique, it was detected that microdeletion of CLCN5 mRNA and splicing abnormality of CLCN5 Cl<sup>-</sup> channel was found in some patients (Morimoto *et al.*, 2001).

Equivalent over saturation of salts in urine of lithiasic patient has a greater tendency to form crystals indicating that urine of stone former may contain substances that promote crystallization. It was found that changes in the amino acid sequence of urinary proteins can cause these to behave like calculus promoting substances (Fernandez *et al.*,

1997). Under physiological conditions several macromolecules related to 1-alpha-1 appear in plasma and urine. One of these inhibitors, uronic acid rich protein (UAP), isolated from stone former, exhibited low inhibiting activity towards calcium oxalate crystallization than derived from the urine of healthy subjects. This suggested that abnormality of inhibitor is present in stone former (Atmani *et al.*, 1996). Precipitation properties of albumin transferring and Tamm-Horsfall mucoprotein and high concentration in stone matrix relative to those in urine suggested that they play a role in stone formation (Fraj, 1989). Later study revealed statistically significant different pattern of Tamm-Horsfall protein in the urine of lithiasic patient and control.

SDS-PAGE of serum proteins has an important role as a diagnostic investigation. The present study is carried out to investigate protein profile in the sera of families indisposed to nephrolithiasis and without any history of this disease in population of northern Punjab in order to find any fraction/s associated with nephrolithiasis. The indication of any such protein fraction in the comparisons of the nephrolithiasis affected and unaffected samples may provide information for its development in the diagnosis and management of the disease.

## MATERIALS AND METHODS

M. Tariq clinical laboratory, Al- Bashir Hospital Sadiqabad provided clinical facilities for blood sampling of affected and control families. Blood samples were processed for serum and later were transported from Sadiqabad to Lahore, while being kept in ice-cooler. Two control and seven affected families were selected for analysis.

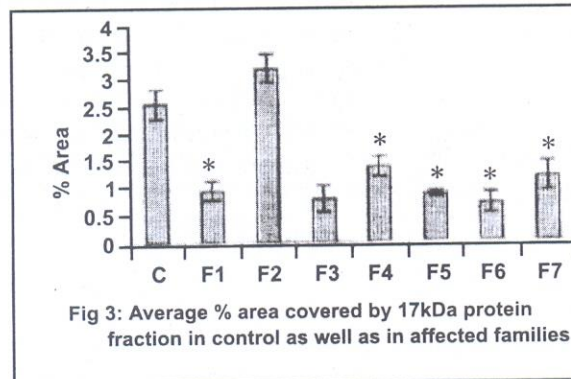
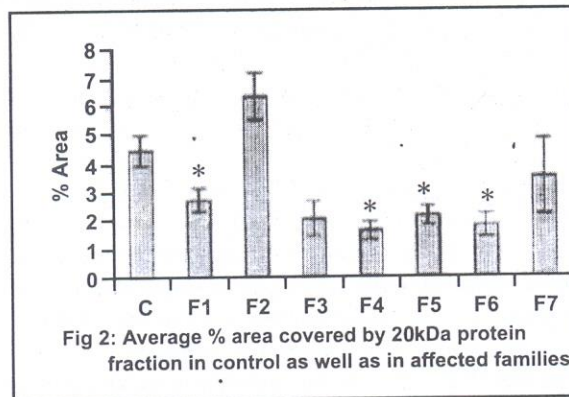
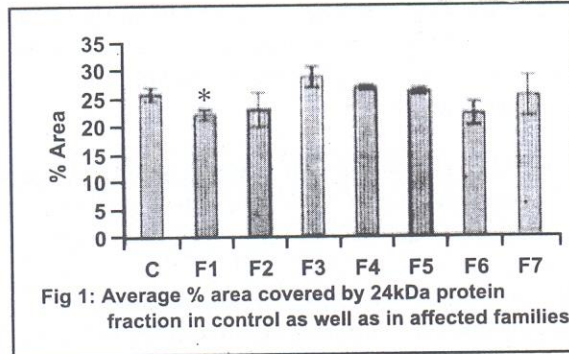
15% Polyacrylamide gel was prepared for studying the low molecular weight protein fractions using the method of Laemmli (1970). Serum was diluted with distilled water and proteins were denatured by heating with loading dye. Protein size marker and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 12 mA and voltage of 150 mV, in a cooling chamber maintained at 4°C until the tracking dye reached the lower end of the gel. Following electrophoresis, the gels were stained with coomassie brilliant blue for a duration of two hours and destained afterwards until the protein fractions of different molecular weights became visible in the form of blue bands on a transparent background.

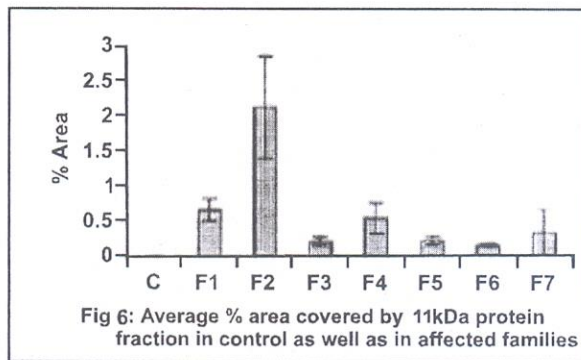
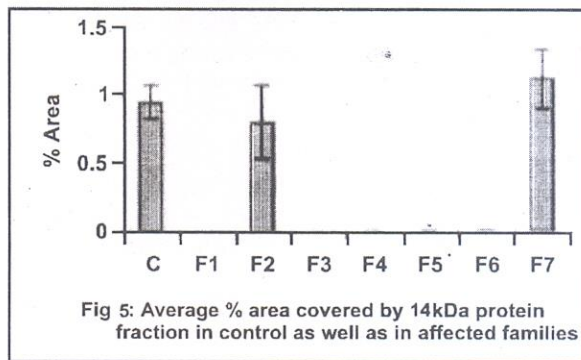
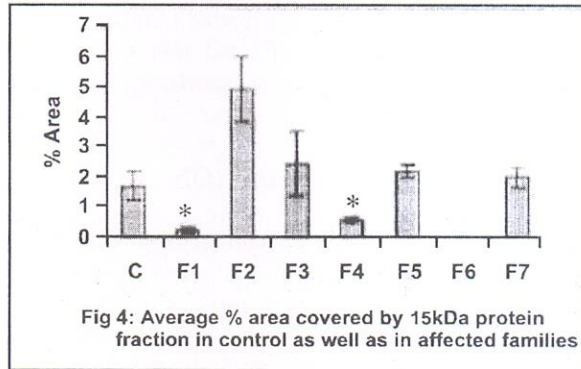
Photographs of stained gels and quantification of separated protein fractions were carried out by Gene Genius Bio-imaging Gel Documentation system that provided the data of molecular weight and percent area covered by each of the fractions. The data was analyzed statistically using Student t-test and employed in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparison among control and affected families suffering from nephrolithiasis.

The detailed analysis of protein profile pattern resulted in five fractions in control families ranging between 24-14kDa and six protein fractions ranging between 24-11kDa in affected families. One fraction of 11kDa that appeared in affected families and which could not be detected in control families, covered average area of  $0.66\pm 0.15$ ,  $2.12\pm 0.73$ ,  $0.20\pm 0.07$ ,  $0.55\pm 0.27$ ,  $0.22\pm 0.06$ ,  $0.15\pm 0.03$  and  $0.33\pm 0.05$  % in affected families No 1 to 7, respectively (Fig 6).

In comparison of control with affected families, protein fraction of 24kDa did not exhibit any significant change. However, non-significant decreases of 15, 12, 15, and 6 % were noticed in families No. 1, 2, 6 and 7 constituting average areas of  $21.98\pm 0.88$ ,  $22.78\pm 3.12$ ,  $21.77\pm 2.05$  and  $24.28\pm 3.57\%$ , respectively, and non-significant increase of 11% in family No. 3 covering average area of  $28.55\pm 1.89\%$ . On the other hand, same fraction exhibited no change in families No 4 and 5 covering average area of  $26.02\pm 0.40$  and  $25.86\pm 0.58\%$ , respectively (Fig .1). Protein fraction of 20kDa exhibited significant decreases of 40, 55, 65, 53 and 61% in family No. 1, 3, 4, 5 and 6 covering average areas of  $2.72\pm 0.42$ ,  $2.03\pm 0.61$ ,  $1.59\pm 0.33$ ,  $2.16\pm 0.33$  and  $1.74\pm 0.41\%$  respectively. Family No. 2 and 7 exhibited non-significant increase, however, of 41 and 21%, covering average areas of  $3.42\pm 1.31$  and  $6.32\pm 0.82\%$ , respectively (Fig.2).

Another protein fraction of 17kDa was found to be markedly decreased in most of affected families, indicating pronounced decreases of 63, 64, 46, 67, 73 and 54% in family No 1, 3, 4, 5, 6 and 7 covering average areas of  $0.94\pm 0.17$ ,  $0.78\pm 0.24$ ,  $1.36\pm 0.18$ ,  $0.85\pm 0.06$ ,  $0.69\pm 0.19$  and  $1.17\pm 0.27\%$ , respectively. Same fraction expressed non-significant increase of 25% in family No. 2 covering average area of  $3.18\pm 0.26\%$  (Fig.3). Another protein fraction of 14kDa that appeared in control families could be observed in families No. 2 and 7 only, indicating non-significant increases of 16 and 18 %, respectively, covering the respective areas of  $0.80\pm 0.27$  and  $1.21\pm 0.22$  %. Protein fraction of 15kDa exhibited variable results when affected families were compared with control family No 2, 3, 5 and 7 exhibiting non-significant increases of 19, 14, 27 and 15% covering average area of  $4.91\pm 1.11$ ,  $2.41\pm 1.06$ ,  $2.15\pm 0.21$  and  $1.95\pm 0.32\%$ , respectively, whereas, significant decreases of 86 and 65% are noticed in family No.1 and 5 covering respective areas of  $0.24\pm 0.09$  and  $0.55\pm 0.11\%$ . However this fraction could not be expressed in family No.6 (Fig.4).





\* Significance at  $p < 0.05$

Inter group comparison of control with affected members of affected families and control with healthy members of affected families reveals the similar results. However, an intra group comparison of affected and healthy members of affected families showed that no significant differences were found in protein fractions within a family.

## DISCUSSION

Nephrolithiasis is a complex multifactorial disease resulting from an interaction between environmental and genetic factors even if the actual contribution of this disease is not yet quantifiable. The high rates of nephrolithiasis among affected families with an increased risk for 1st degree relatives confirm the heredity for nephrolithiasis (Scott *et al.*, 1998). In present study the most important result is that  $\beta$ -2-microglobulin (11kDa) was absent in controls and appeared in affected families. The urinary excretion of  $\beta$ -2-microglobulin increased during acidosis and alkalosis in patients with renal stones. The tubular proteinuria that could be provoked during acidosis and alkalosis was considered to be secondary to changes in the acid base status and may indicate a renal tubular defect (Backman *et al.*, 1996). In present study, serum  $\beta$ -2-microglobulin that appeared only in affected families, may have a connection with increased excretion of  $\beta$ -2-microglobulin in urine of nephrolithiasis patient. Study of serum and urine  $\beta$ -2-microglobulin concentration in patients with urolithiasis that with and without pyelonephritis confirmed increased concentration of  $\beta$ -2-microglobulin and suggested that serum  $\beta$ -2-microglobulin concentration may be of value in evaluation of glomerulus filtration rate (Pupek, 1993).

Renal stone disease, which affected 12% male and 5% female, occur as inherit disorder in patients (Thakker, 2000). As oxaluria and calciuria have prominent role in stone formation, so any gene that influences their excretion can be considered as prime candidate in nephrolithiasis. In a work on X-linked recessive nephrolithiasis (XRN), low molecular weight proteinuria was indicated in affected males, the excretion of  $\alpha$ -1-microglobulin exceeded normal by 3-14 folds, of  $\beta$ -2-microglobulin exceeded normal by 100-300 folds and retinol binding protein exceeded normal by 1000-3000 folds (Reinhart *et al.*, 1995). Study of two dimensional electrophoretic maps of matrix protein extracted from calcium oxalate and uric acid calculi revealed majority of protein maps were note worthy for the presence of a low molecular weight pattern (M.W. less than 17.5 kDa) not seen in associated with normal urinary maps. The significance of this pattern was not known but the result of degradation of larger protein (Jones and Resnick, 1990). In our present study apolipoprotein (17kDa) and  $\alpha$ -lact albumin (14kDa) were present in controls but absent in majority of the affected families It is suggested that these parameters may be worked on larger scale so that further investigation may provide better understanding on genetic interrelationship in nephrolithiasis.

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