Fabry Disease: Screening and Analysis of the Associated Clinical Manifestations in Patients Attending Dialysis and Nephrology Clinics in Durban, South Africa

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Abstract

Background

Fabry disease is inherited in an X-linked manner in which the mutated gene inhibits the functioning of the alpha-Galactosidase-A enzyme causing a deficiency or absence of the enzyme, characterising it as a progressive, lysosomal storage disorder. Subsequently, the accumulation of globotriaosylceramide (Gb3) in the lysosomes causes damage to tissues and major organs. Fabry nephropathy is one of the major organ complications caused by Fabry disease resulting in end-stage kidney disease. To our knowledge, no research has been conducted to determine the association between Fabry disease, its clinical manifestations, and chronic kidney disease in Durban.

Methods

This study was a prospective, quantitative study. A cohort of 200 male patients with chronic kidney disease (CKD stage 2-5D) was enrolled. A control group of 14 healthy males was also enrolled for this study. The ELISA technique was employed to determine the alpha Gal-A enzyme concentration levels in plasma. A questionnaire using the MSSI scoring system was presented to the participants to identify clinical manifestations. The SPSS Version 27 (IBM, New York, USA) was used to analyse the data.

Results

A cut-off value for the alpha Gal-A enzyme concentration levels of < 500pg/ml was calculated. A total of 17 participants from the patient group (n = 11) and the control group (n = 6) displayed alpha-Gal-A enzyme levels < 500pg/ml. The univariate regression analysis revealed, statistically significant association between alpha-Gal levels < 500pg/ml and age (p = 0.007), heat or cold intolerance (p = 0.049), hypertension (p < 0.001) and eGFR (p < 0.001). MSSI scores displayed a negative association (p = 0.001). The multivariate regression analysis showed that age and MSSI scores retained their significance when eGFR was excluded as a variable, however, with the inclusion of eGFR as a variable, none of the variables retained their significance.

Conclusion

Fabry disease is suspected in 17 participants with alpha-Gal levels of < 500pg/ml. The cause of CKD nephropathy raises interest as conditions such as FSGS have been associated with FD. The low levels of the alpha-Gal enzyme and the presentation of the clinical manifestations can be utilised as preliminary findings. Confirmatory tests such as DNA analysis or Gb3 and GL3 analysis should be performed to confirm the diagnosis.
Introduction

Fabry disease (Online Mendelian Inheritance in Man #301500) (FD, OMIM) is classified as a lysosomal storage disorder. Lysosomal storage disorders (LSD) are a group of disorders that are inherited or acquired. The disruption of the primary function of the recycling and disposal centres of the lysosome due to errors with the encoding of different lysosomal proteins, lysosomal enzymes, and lysosomal membrane proteins is the main feature of LSD (1). Fabry disease is defined as a complex multisystem disease with non-specific signs and symptoms and is the second most frequent disorder of this type after Gaucher’s disease (2). The characteristic feature of Gaucher’s disease is the presence of lipid-laden reticuloendothelial cells present in the spleen, liver, and bone marrow contributing to symptoms such as hepatosplenomegaly and pancytopenia (3). Fabry disease, also known as Alpha-Galactosidase A Deficiency or Anderson-Fabry disease, is the deficiency of the alpha galactosidase-A (α-Gal A) enzyme. The monogenic disease is inherited in an X-linked manner. The alpha-galactosidase A (GLA) gene is situated on the Xq22.1 position on the X chromosome, affecting all hemizygous males; their daughters become heterozygous carriers, and their sons are non-carriers and remain unaffected (4). An incomplete functioning or deficiency of the alpha-galactosidase A enzyme results in a systemic, intracellular accumulation of complex glycosphingolipids, mainly globotriaosylceramide (Gb3) or the water-soluble deacylated Gb3 known as globotriaosylsphingosine (lyso-Gb3) causing progressive damage to tissues and major organs, including amongst others, the heart, brain, vascular endothelium and kidneys led to end-stage renal disease (5). Ethnic preference has not been observed in FD. Due to the low incidence rate of the condition, the prevalence rate can only be estimated as ranging from 1:40 000 men to 1:117 000 live births (5). The prevalence in live births has significantly increased with atypical mutations of the disease included, with statistics varying between 1:2900 to 1:3900 (6).

Early symptoms manifesting during childhood and adolescence include pain in the extremities, angiokeratomas, tinnitus, and anhidrosis. Disease progression ultimately results in left ventricular hypertrophy (LVH), stroke, proteinuria, and renal failure. Complications of the heart, cerebrovascular, or kidneys become pronounced after the age of 30, reducing mortality by 20 years (7). Under- or misdiagnosis of the disease during later manifestations is common due to the non-specific nature and the mimicking of symptoms associated with diabetes and hypertension (8).

Diagnosing Fabry disease in its early stages has proved challenging due to the variability of symptoms, thereby increasing the frequency of organ involvement in the later stages of the disease. Chronic kidney disease (CKD) remains as one of the main characteristics of Fabry disease. The deposition and accumulation of Gb3 occur first in the glomeruli and progress to various areas of the nephron, including the mesangial, and interstitial cells, podocytes, cells of the proximal and distal tubules, and loop of Henle, and the vascular endothelial cells, including the smooth muscle cells (9). Proteinuria is an early indication in the detection of the disease in both males and females and is usually a significant clinical manifestation of renal involvement (10). The appearance of proteinuria occurs mainly during the second to third decades of life. However, it is also shown to be evident in male and female adolescents as well as boys as young as six years old (10).
Diagnosis of FD in males requires observing low levels or absence of the alpha-galactosidase-A enzyme activity in leukocytes, plasma, or fibroblasts and increased levels of Gb3 and lyso-Gb3 concentrations in plasma and urine. Pathogenic mutations can be assessed by genetic analysis (11). The absence of residual enzyme activity in males is categorised as a severe classical phenotype, where characteristic FD symptoms manifest and progress into more severe symptoms later in life and affect multiple organs. The non-classical phenotype is milder in males where residual enzyme activity is evident. Patients are less severely affected, and only one organ is affected later in life. Heterozygous females can present with normal alpha-galactosidase levels due to skewed X-inactivation (as per Lyons hypothesis whereby the process of the X chromosome is rendered inactive) and therefore are not reliably diagnosed by enzymatic assay. In such cases, molecular analysis is required (12).

The Kidney Disease Improving Global Outcomes (KDIGO) foundation recommends testing patients with chronic kidney disease when biopsies are not performed and there is no definitive cause of nephropathy (13). The European Best Practice Guidelines (EBPG) recommend testing males under 50 years of age with chronic kidney disease with no definitive diagnosis (8). Although there have been documented cases of Fabry disease in South Africa, the prevalence has not been established.

Treatment for Fabry disease currently comprises two forms of recombinant enzyme replacement therapy (ERT): Agalsidase-alpha produced in human fibroblasts and agalsidase-beta produced in Chinese hamster ovary cells (14). Both forms of treatment with enzyme replacement require biweekly administration. Oral chaperone therapy is a relatively new form of treatment; however, it is effective in patients with specific mutations of the disease (15). Novel gene therapies are in the clinical trial phase and are producing promising results through in vivo and ex vivo procedures (16). Although reports have shown that after a short course of enzyme replacement therapy patients showed improvements in pain relief, ability to sweat, and quality of life, there were no improvements to the cerebrovascular and renal damage (17).

The Fabry Outcome Survey (FOS), established in 2001, is an international database designed to enhance the clinical management of patients diagnosed with Fabry disease (6). The data collected on the FOS provides information on the safety and efficacy of enzyme replacement therapy as well as the natural history of Fabry's disease. The FOS patient report confirmed that as of January 2019, 3855 patients were enrolled in the FOS from 26 countries, which is a 10% increase from January 2018.

In this prospective, quantitative study, 200 male patients with chronic renal failure were tested for low levels or absence of the alpha-Galactosidase-A enzyme. Clinical manifestations were assessed and scored using the Fabry Outcome Survey Mainz Severity Score Index (FOS-MSSI). Results displaying low levels or absence of the Alpha-Galactosidase-A enzyme in conjunction with high scores in the assessment of their clinical manifestation will establish an association between Fabry disease and chronic renal failure. Therefore, this study aims to demonstrate that early testing for Fabry disease is required when the diagnosis of chronic kidney disease is unconfirmed. This can ultimately retard or prevent damage to all major organs.
Materials And Methods

Study Participants

A cohort of 200 male patients with chronic renal failure was selected consecutively from three provincial hospitals, viz., Addington Hospital, Inkosi Albert Luthuli Central Hospital, and St. Aidan’s Hospital. Two patients were confirmed prior to this study with Fabry disease and were enrolled as positive controls. The patients enrolled on the study were male patients receiving hemodialysis, peritoneal dialysis, and pre-renal patients. Male patients were diagnosed with chronic renal failure, i.e., stage 5 CKD (eGFR < 15ml/min) and were receiving HD as outpatients. Participants receiving peritoneal dialysis with stage 5 CKD were also considered. Pre-dialysis candidates with stage 2 CKD (eGFR 60-89ml/min) to stage 4 CKD (eGFR 15-29ml/min) were also enrolled. The research study was explained to all patients who were interested in participating and a consent form was signed before any investigations were performed. Patients were informed that participation was voluntary and they were entitled to withdraw from the study at any point without consequences regarding their treatment. An additional 15 healthy male participants with no renal impairment (eGFR > 90ml/min - Stage 1 CKD) were enrolled as a control group. A questionnaire employing the Mainz Severity Score Index (MSSI) was provided to allow the participants to identify any of the clinical manifestations of Fabry disease. The total sample size of participants was 215. However, one control participant unknowingly had diminished kidney function with an eGFR of 37ml/min/1.73m$^2$, indicating stage 3 CKD and had to be excluded from the study. The participant was counselled and advised to consult his doctor for further examination. Ethical approval (IREC) was obtained from the Durban University of Technology before any investigative work was commenced. All methods were performed in accordance with the relevant guidelines and regulations.

Blood Sampling and Storage of Samples

Blood samples of haemodialysis patients were drawn pre-dialysis from their dialysis access. The 5ml blood sample was taken using an EDTA tube from the patient's dialysis access after consent was obtained. Patients from the pre-renal and CAPD clinics were interviewed, and once consent was obtained, a blood sample of 5mls was taken from the antecubital vein using an EDTA tube. Control participants were interviewed, and after consent was obtained, blood samples were taken from the antecubital vein. A 5ml blood sample was taken using a serum separator tube (SST) and transported at room temperature to a pathology laboratory for testing of participants’ urea, creatinine, and eGFR levels. A second blood sample of 5mls was taken from the same site using an EDTA tube which was centrifuged and stored for analysis.

Patient anonymity was maintained by using reference numbers to identify samples. The blood samples were transferred into 15ml conical bottom centrifuge tubes and centrifuged at 4000rpm for 10 minutes at 5°C to separate the plasma from the blood components. A volume of 1ml of plasma was transferred into microcentrifuge tubes and labelled according to the corresponding patient reference numbers. The samples were stored at -80°C. These were prepared in duplicate. In addition, the buffy coat layer
containing the polynuclear cells was transferred into microcentrifuge tubes and stored at -80°C for further future genetic studies.

**Enzyme-Linked Immunosorbent Assay**

The Enzyme-Linked Immunosorbent Assay (ELISA) technique was employed to determine the levels of Alpha-Gal-A enzyme concentration levels in the plasma samples.

**Statistical Analysis**

The results were analysed using logistic regression analysis and were calculated using the IBM SPSS version 27 (IBM, New York, USA). A univariate logistic regression analysis was implemented to determine the association between alpha Gal-A enzyme levels < 500pg/ml and the individual clinical manifestations. A multivariate logistic regression analysis was performed subsequently to determine the association between alpha Gal-A enzyme levels < 500pg/ml and variables that presented a significant result in the univariate logistic regression analysis. The variables of the patient population and the control group were also analysed using descriptive statistical analysis. A p-value of < 0.05 was used to indicate the significance between the variables and the alpha Gal-A enzyme concentration levels < 500pg/ml. Scatter plots were constructed to provide a graphical representation of variables in comparison to the alpha Gal-A enzyme concentration levels. The MSSI scores were calculated for each participant individually.

**Results**

Using the control group as a benchmark for the cut-off value, we determined the average control value was 756 ± 675. Based on this, 756pg/ml should have been employed as the cut-off value. However, it is established that Fabry disease is unlikely with high levels of the alpha-Gal-A enzyme and we concluded that a value of 500pg/ml was acceptable to utilise as a cut-off value.

In our study, a total of 11 patients exhibited concentration levels of < 500pg/ml (n = 11). Table 1 represents the variables that were analysed for patients with alpha-Gal levels < 500pg/ml using the univariate logistic analysis. Significance was evident with clinical parameters such as heat or cold intolerance (p = 0.049) and hypertension (p < 0.001). Demographic parameters such as age were also significant, with p = 0.007. The mean age of all participants with levels < 500pg/ml was 30.5 years. The eGFR showed significance with p < 0.001. The MSSI scores displayed a negative significance where p = 0.001. The remaining variables were not significant and were not considered further. We further analysed the variables by employing multivariate logistic analysis (Table 2). In Table 2, the significant variables from the univariate analysis were analysed and revealed no significance between the variables and alpha-Gal levels < 500pg/ml. To further analyse the significance, the eGFR variable was removed from the multivariate logistic analysis (Table 3), and this revealed there remained no association with hypertension (p = 0.057) and heat/ cold intolerance (p = 0.206). An association between age and alpha-Gal levels < 500pg/ml remained where p = 0.044. A negative significance was still evident with the MSSI scores with p = 0.027.
Table 1
Logistic regression analysis for alpha Gal-A concentration < 500pg/ml

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ODDS RATIO (EXP B)</th>
<th>CONFIDENCE INTERVAL (95%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat/ cold intolerance</td>
<td>0.226</td>
<td>[0.051–0.994]</td>
<td>0.049</td>
</tr>
<tr>
<td>Angina</td>
<td>0.000</td>
<td>.000</td>
<td>0.999</td>
</tr>
<tr>
<td>Skin (angiokeratoma)</td>
<td>0.500</td>
<td>[0.063–3.956]</td>
<td>0.511</td>
</tr>
<tr>
<td>Ringing in ear</td>
<td>0.609</td>
<td>[0.076–4.866]</td>
<td>0.640</td>
</tr>
<tr>
<td>Vertigo</td>
<td>0.000</td>
<td>.000</td>
<td>0.999</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.166</td>
<td>[0.067–0.409]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.953</td>
<td>[0.920–0.987]</td>
<td>0.007</td>
</tr>
<tr>
<td>Anhidrosis</td>
<td>1.159</td>
<td>[0.318–4.220]</td>
<td>0.823</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.463</td>
<td>[0.132–1.627]</td>
<td>0.230</td>
</tr>
<tr>
<td>Race</td>
<td>0.650</td>
<td>[0.324–1.302]</td>
<td>0.224</td>
</tr>
<tr>
<td>Migraine</td>
<td>0.748</td>
<td>[0.092–6.074]</td>
<td>0.786</td>
</tr>
<tr>
<td>MSSI Score</td>
<td>0.911</td>
<td>[0.863–0.961]</td>
<td>0.001</td>
</tr>
<tr>
<td>Abdominal cramping</td>
<td>0.000</td>
<td>.000</td>
<td>0.998</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.000</td>
<td>.000</td>
<td>1</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.000</td>
<td>.000</td>
<td>0.998</td>
</tr>
<tr>
<td>Initial eGFR</td>
<td>1.035</td>
<td>[1.019–1.051]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2
Multivariate Regression Analysis for Significant Variables including eGFR

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ODDS RATIO (EXP B)</th>
<th>CONFIDENCE INTERVAL (95%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSI Score</td>
<td>0.982</td>
<td>[0.908–1.062]</td>
<td>0.651</td>
</tr>
<tr>
<td>Initial eGFR</td>
<td>1.022</td>
<td>[0.997–1.047]</td>
<td>0.089</td>
</tr>
<tr>
<td>Age</td>
<td>0.976</td>
<td>[0.941–1.011]</td>
<td>0.177</td>
</tr>
<tr>
<td>Heat/ Cold Intolerance</td>
<td>0.328</td>
<td>[0.065–1.650]</td>
<td>0.176</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.519</td>
<td>[0.158–1.702]</td>
<td>0.279</td>
</tr>
</tbody>
</table>
Table 3
Multivariate Regression Analysis for Significant Variables excluding eGFR

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ODDS RATIO (EXP B)</th>
<th>CONFIDENCE INTERVAL (95%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSI Score</td>
<td>0.936</td>
<td>[0.883–0.992]</td>
<td>0.027</td>
</tr>
<tr>
<td>Age</td>
<td>0.966</td>
<td>[0.934–0.999]</td>
<td>0.044</td>
</tr>
<tr>
<td>Heat/ Cold Intolerance</td>
<td>0.366</td>
<td>[0.077–1.736]</td>
<td>0.206</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.359</td>
<td>[0.124–1.033]</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Table 4
Summary of variables showing p-values using various logistic regression analysis

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>UNIVARIATE (p-value)</th>
<th>MULTIVARIATE (WITH eGFR) (p-value)</th>
<th>MULTIVARIATE (WITHOUT eGFR) (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat/cold intolerance</td>
<td>0.049</td>
<td>0.176</td>
<td>0.206</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&lt; 0.001</td>
<td>0.279</td>
<td>0.057</td>
</tr>
<tr>
<td>Age</td>
<td>0.007</td>
<td>0.177</td>
<td>0.044</td>
</tr>
<tr>
<td>MSSI score</td>
<td>0.001</td>
<td>0.651</td>
<td>0.027</td>
</tr>
<tr>
<td>eGFR</td>
<td>&lt; 0.001</td>
<td>0.089</td>
<td></td>
</tr>
</tbody>
</table>

A bar graph (Fig. 1) displaying the standard mean of error was constructed to demonstrate the average alpha-Gal-A concentration levels of the CKD stage 2–5 of the patient group. Patients with eGFR > 60ml/min (CKD stage 2) displayed lower levels of alpha-Gal levels. Patients with eGFR < 15ml/min/1.73m² (CKD stage 5) demonstrated higher alpha Gal-A levels of 4000- >5000pg/ml.

The alpha Gal-A enzyme concentration levels < 500pg/ml were assessed using a scatter plot graph (Fig. 2). A total of 11 patients exhibited concentration levels of < 500pg/ml (n = 11).

Alpha Galactosidase-A enzyme concentration levels of control participants (n = 15).

The levels of alpha galactosidase-A enzyme for control participants. A scatter plot was constructed to represent the concentration levels of alpha Gal-A enzyme levels (Fig. 3). Patient 13 had to be excluded from the study due to his eGFR revealing Stage 3 CKD.
A graphical representation using a scatter plot was constructed to find a correlation between the MSSI score of the patients and the alpha Gal-A concentration levels < 500pg/ml (Fig. 4). There was a negative significance (p = 0.001) using the univariate analysis between the MSSI scores and alpha Gal-A levels < 500pg/ml. The multivariate analysis with eGFR as a variable showed no significance with MSSI scores (p = 0.651), while the multivariate analysis excluding eGFR as a variable showed significance with MSSI scores where p = 0.027 (Table 4).

A scatter plot graph was constructed to illustrate the association between the MSSI scores of the control group and the alpha-Gal-A concentration levels (Fig. 5). Participants in the control group did not report any clinical manifestations.

The bar graph (Fig. 6) depicts the diagnoses of the patient group with alpha-Gal levels < 500pg/ml (n = 11). Systemic lupus erythematosus (SLE) was diagnosed in 3 patients (27%), focal segmental glomerular sclerosis (FSGS) was diagnosed in 6 patients (54%), HIV-associated nephropathy (HIVAN) was diagnosed in 1 patient (9%) and minimal change disease (MCD) was diagnosed in 1 patient (9%).

A bar graph was constructed (Fig. 7) to depict the average alpha-Gal levels of the diagnosis of patients with alpha-Gal levels < 500pg/ml.

**Discussion**

The deficiency or absence of alpha-Galactosidase-A enzyme can confirm the diagnosis of Fabry disease in males. However, confirmatory testing using GLA sequencing is required. The prevalence of Fabry disease in patients with end-stage renal disease (CKD stage 5D) is estimated at around 0.12% (18). It is estimated that the prevalence of Fabry disease among males undergoing haemodialysis (CKD 5D) is 0.59–1.8% (19). In this study, patients (n = 11) with alpha-Gal-A enzyme levels < 500pg/ml were all in stage 2 CKD with eGFR of > 60ml/min/1.73m². The univariate logistic regression analysis revealed that eGFR in patients with alpha-Gal-A levels < 500pg/ml was significant with p = < 0.001. To verify independent association, a multivariate logistic regression analysis was employed, which revealed no significance (p = 0.089) when analysed against variables which showed significance in the univariate analysis. None of the patients with alpha-Gal levels < 500pg/ml had commenced with dialysis. Nagata et al. (2021) concluded in their study that FD prevalence was higher in male patients with CKD stages 1–5 than in those with CKD stage 5D (19). In this study, the MSSI scores of the patient group and control group indicated significance (p = 0.001). However, this is questionable. Lower MSSI scores should suggest a lesser possibility of FD. The median MSSI score of the patient group was 8. The control group reported no symptoms, and therefore the score was 0. A score of ≤ 18 is classified as mild FD and the highest score was 12.5 of which only one patient obtained.

The age of the patients with low alpha Gal-A levels Fabry disease in our study showed a significance where p = 0.007 (confidence interval [0.920–0.987]), where the average age of the patients CKD 2–5 was
27 years, and the average age of the control group with low levels < 500pg/ml was 45 years. Further analysis using the multivariate logistic analysis revealed that age with eGFR included as a variable (Table 2) had no significance with p = 0.177. However, when eGFR was removed as a variable (Table 3), age retained its significance with p = 0.044. In reports by Eng et al., the Fabry Registry was shown to have age ranges of enrolled patients as young as a newborn, with the diagnosis being confirmed in the prenatal stages and patients as old as 85 years (20).

In our study, only 11% (n = 2) of patients with alpha-Gal-A levels < 500-g/ml disclosed heat or cold intolerance, however, there was a significance with a p-value of 0.049. One patient reported heat intolerance and anhidrosis, which typically manifest together with Fabry disease. The second patient reported intolerance to cold. In a study by Bashourum et al., one of the most frequently reported symptoms was intolerance to heat or cold (21).

Renal impairment due to FD could also contribute to the development of hypertension and impact blood pressure control. In a study conducted by Dincer et al. (2022), the results demonstrated that all blood pressure measurements were lower in the patients with FD than in the control group. They concluded that a decrease in heart rate variability, rather than an increase in blood pressure variability, might be an early indicator of FD (22). Another study conducted by Rossi et al. also demonstrated a lower prevalence of hypertension in patients with ESKD, pre-renal, and patients with transplants (23). In our study, there was a significance (p = < 0.001) between hypertension and patients with alpha-Gal-A levels < 500pg/ml when the univariate analysis was employed. However, hypertension did not retain its significance when we employed the multivariate analysis of whether the eGFR was included or not where p = 0.279 and p = 0.057, respectively. In the patient group, four patients reported hypertension; however, it is important to note that the patients were in stage 2 CKD, and the cause of hypertension was unknown. Whether the hypertension is due to low levels of alpha-Gal-A enzyme levels or CKD requires further investigation.

In this study, of the 11 participants in the patient group, focal segmental glomerular sclerosis (FSGS) was diagnosed in 6 patients (54%), systemic lupus erythematosus (SLE) was diagnosed in 3 patients (27%), HIV-associated nephropathy (HIVAN) was diagnosed in 1 patient (9%) and minimal change disease (MCD) was diagnosed in 1 patient (9%).

There have been reports of the association between FSGS and FD as far back as 2005 when Svarstad et al. (2005) described the presence of FSGS and vascular changes in a male and female with FD. He concluded that FSGS has the potential role as a marker of progressive renal disease in some Fabry patients (24). Fabry disease and FD share similar pathophysiological characteristics since both cause podocyte damage. Recent studies by Hasbal et al. (2020) revealed that alpha-Gal-A enzyme levels in patients with FSGS were lower than in patients on haemodialysis (2.88 ± 1.2 mmol/L/h versus 3.79 ± 1.9 mmol/L/h, p < 0.001) (25). In our study, 23 patients (11.5%) were diagnosed with FSGS. In the haemodialysis group (CKD stage 5D), only one patient (1.7%) was diagnosed with FSGS with alpha-Gal levels > 5000pg/ml. In the pre-renal group (CKD stage 1–5), 22 patients (22%) were diagnosed with FSGS. Only one patient (4.5%) (CKD stage 2) from this group had a level of 3271pg/ml. The remaining patients
(n = 21) (95%) had levels < 1600pg/ml. This is comparable with studies conducted by Hasbal et al., where it was concluded that patients with FSGS had lower alpha-Gal activity than patients receiving haemodialysis (Hasbal et al. 2020). Out of the 21 patients, 6 patients (29%) had alpha-Gal levels below the cut-off of 500pg/ml with an average alpha-Gal level of 476pg/ml.

In this study, systemic lupus erythematosus (SLE) was diagnosed in 3 patients (27%) with low alpha-Gal enzyme levels < 500pg/ml. All 3 patients had eGFR values > 60ml/min/1.73m². Overlapping organ involvement is evident in the course of FD and SLE (Kiykim et al. 2020). The accumulation of Gb3 causes alterations in the lymphocyte cell membranes enabling an environment suitable for autoimmune disease (26). The presence of zebra bodies during histological examination is usually associated with FD. However, a study conducted by Manabe et al. (2021) biopsied five patients with lupus nephritis during hydroxychloroquine treatment. Zebra body formation and kidney phospholipidosis were evident in the biopsies. None of the patients had clinical manifestations of FD or any family history of FD. No genetic studies were performed to confirm the diagnosis of FD. A comparison of the number and size of the zebra bodies to FD confirmed a likely diagnosis of hydroxychloroquine-associated phospholipidosis (27). SLE-associated autoantibodies have been reported in FD. In a study by Kiykim et al. (2020), 76 juvenile SLE patients (mean age 16 ± 3.3 years; range, 8 to 23.5 years) were tested for FD by GLA sequencing. There were no positive cases found (28). In our study, the median MSSI score of the 3 patients diagnosed with SLE was 8.3. The median age was 35.3 years. The mean alpha Gal level of the 3 patients with SLE was 453pg/ml. Hearing loss in both ears was reported by one patient with SLE.

In our study, only one patient (9%) presented with HIV-associated nephropathy (HIVAN) with alpha-Gal levels < 200pg/ml. There is little that is known regarding the association between FD and HIVAN. It has been established; however, that sphingolipid accumulation is evident in glomerular diseases such as HIVAN (Hasbal et al. 2020). In the present study, the patient was a 50-year-old male who reported hypertension and anhidrosis. His MSSI score was 10, and eGFR > 60ml/min/1.73m².

Only 1 patient (9%) was diagnosed with minimal change disease in this current study. Minimal-change disease (MCD), otherwise known as lipoid nephrosis or nil disease, is the most common cause of idiopathic nephrotic syndrome in children (29). It is characterised by intense proteinuria leading to oedema and intravascular volume depletion. Light microscopic findings reveal normal glomeruli or mild mesangial proliferation with negative immunofluorescence and no immune complex deposition, while electron microscopy typically demonstrates diffuse effacement of the epithelial cell foot processes (30). A nonsense mutation has been reported in FD with nephrotic syndrome developing secondary to minimal change disease (31). In the present study, the patient is an 18-year-old male with CKD stage 2 and eGFR > 60ml/min/1.73m². His MSSI score was 8; however, alpha-Gal levels were 185pg/ml. The patient did not present with any other clinical manifestations of FD.

Limitations and Challenges
There were several challenges and limitations in this study. The patient sample size should have been larger owing to the statistics indicating the low prevalence of FD. The study required only males to be enrolled. The male population in the haemodialysis units from the 3 provincial hospitals could have been larger. The patient numbers at the renal clinics were limited due to the COVID-19 lockdown restrictions; therefore, enrolment and blood sampling was delayed. Visitation as the principal investigator was restricted by the hospital management due to lockdown regulations, delaying the enrolment process. The research was being conducted concurrently at the renal clinic, and patients were hesitant to provide blood samples as Covid was a major contributing limiting factor throughout the entire process of the research study. A larger control group of healthy males would have been more effective for better outcomes; however, the sample size for both groups was approved by the supervisors and statistician.

Limitations of the study included analysing only the alpha-Gal-A enzyme concentration levels. Also, since this was a cross-sectional study, the patients’ condition and presentation of clinical symptoms were documented for that day.

**Declarations**

- Ethics approval and consent to participate:
  - Ethical approval was obtained from Institutional research ethics committee (IREC) from the Durban University of Technology (Ethics approval number: IREC 143/19). Each participant in the study signed an informed consent following a counselling session on the study and before any investigative work commenced.

- Consent for publication:
  - This is part of ethical approval. No identifiable material is included in the manuscript. Hence, no further consent for publication is required.

- Availability of data and materials:
  - The datasets generated and/or analysed during the current study are not publicly available due confidentiality agreement, but are available from the corresponding author on reasonable request.

- Competing interests:
  - All authors declare that there are no competing interests.

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  - Durban University of Technology
• Authors' contributions
  ○ J.S. writing of the research protocol, patient recruitment, laboratory experiments and data analysis and writing of the manuscript
  ○ S.B. supervision of the research protocol and correction of the manuscript
  ○ A.A. Supervision of the research protocol, supervision of patient recruitment, laboratory experiments, and assistance with data analysis and correction of the manuscript

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Figures

![AVERAGE eGFR OF CKD STAGE 2-5 vs ALPHA GAL CONCENTRATION](image)

**Figure 1**

Bar graph showing standard error of the mean depicting average alpha Gal-A levels of CKD stage 2-5
Figure 2

Alpha Gal-A enzyme concentration levels <500pg/ml (y axis), patient (x axis)

Figure 3

Alpha Gal-A enzyme concentration levels (y-axis), control (x-axis)
**Figure 4**

Alpha Gal-A enzyme concentration levels <500pg/ml (y-axis), MSSI score (x- axis)- patient

**Figure 5**

MSSI score vs alpha-Gal concentration -Control group
Figure 6

Bar graph depicting the cause of nephropathy in patients with alpha-Gal levels <500pg/ml
Figure 7

Bar graph depicting the average alpha-Gal levels for the individual cause of nephropathy in patients with alpha-Gal levels < 500pg/ml