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Coeliac disease in a South African paediatric type 1 diabetes mellitus clinic

Investigator:

Carla Basson

MBChB (Medunsa), BSc (Stellenbosch), FCPaed (SA)

Student no:

0311281Y

Degree:

MMed (Paed)

Supervisors:

Tim De Maayer

MBBCh (Wits), FCPaed (SA), MMed (Paed), Cert Gastronterol (SA) Paed

Nicole van Wyk

MBBCh (Wits) DCH(SA) FCPaed (SA)

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University of the Witwatersrand, Johannesburg

School of Medicine

SENATE PLAGIARISM POLICY

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I hereby declare the following:

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- I confirm that all the work submitted for assessment for the above course is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

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Coeliac Disease in a South African Paediatric Type 1 Diabetes Mellitus clinic.

Carla Basson, Tim De Maayer, Nicole van Wyk

Introduction:

Coeliac Disease (CD) is an immune mediated systemic condition, triggered by diets containing gluten and related prolamines in genetically susceptible individuals. There is an increased prevalence of CD in Type 1 Diabetes Mellitus (T1DM) with both conditions associated with HLA-DQ2 and DQ8 haplotypes. Multiple studies report variable prevalence rates of CD in T1DM ranging between 1.6-16% with a mean value ranging between 4.5-8%.

Objectives:

To determine the prevalence of CD in a T1DM paediatric population presenting to a single tertiary academic hospital in Johannesburg.

Methods:

A retrospective audit of the records of T1DM children who were seen and screened for CD from January 2014 until March 2017.

Findings:

A total of 76 T1DM patients were included in this study. Eleven had positive serology results for CD. Of the positive serology group nine patients had duodenal biopsies. Six patients (8%) had Marsh grading scores of two or three confirming CD. The majority of biopsy proven CD patients were asymptomatic with normal anthropometry. There were significant differences in prevalence among different ethnic groups.

Conclusions:

The authors have found evidence to support international guidelines to screen all T1DM paediatric patients for CD in South Africa. Prevalence rates of CD in this T1DM group was consistent with other international studies. There is a need for larger group studies and exploring the role of different ethnicities in South Africa.

Introduction

Coeliac Disease (CD) is an immune mediated systemic condition in genetically susceptible individuals, triggered by diets containing gluten and related prolamines. There is an increased prevalence of CD in Type 1 Diabetes Mellitus (T1DM) with both conditions associated with Human Leukocyte Antigen (HLA) DQ2 and DQ8 haplotypes.(1–3) Multiple studies report variable prevalence rates of CD in T1DM ranging between 1.6-16% with a mean value ranging between 4.5-8%.(1,4–7)

Long term complications of untreated CD include failure to thrive, fatigue, delayed puberty, poor dental enamel, osteopenia, osteoporosis, bone fractures, dermatitis herpitiformis and infertility. There is an increased risk of developing intestinal malignancy, specifically small bowel carcinoma, Hodgkin's and non-Hodgkin's lymphoma.(8) CD is thought to have an adverse effect on growth of T1DM patients if left untreated.(5,7) When CD occurs in combination with T1DM, studies have shown an increased risk of retinopathy after 10 years, low bone mineral density, and an earlier increase in urinary albumin excretion which predisposes to nephropathy. There is also the increased occurrence of other autoimmune conditions.(9–15)

CD is not always considered in those of non-European descent and underestimated in developing countries due to lack of awareness.(16) Recent studies in developing countries (Southern Asia, the Middle East, North West and East Africa and South America)where the staple diet includes wheat have shown that the prevalence of CD are similar to Western countries.(3) Currently there is minimal data available on CD in Africa, particularly South Africa. It has recently been found that only a small percentage of children with CD present with classic gastrointestinal features. Many patients present with non-gastrointestinal signs and symptoms and often are asymptomatic, especially in T1DM.(6,8,15,17,18)

The international Society for Paediatric and Adolescent Diabetes (ISPAD) along with many other international guidelines recommend routine screening for CD in all T1DM patients regardless of absence of signs or symptoms.(1,4,5,17,19–22) . Screening should be done at the time of diagnosis of diabetes, followed by screening two and five years thereafter. More frequent screening is suggested if the child is symptomatic for CD or has a first degree relative with CD.(19)

Tests available for screening and diagnosing CD include CD specific antibody tests or HLA testing followed by duodenal biopsies if serology is suggestive. Serology tests recommended by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) include antibodies against tissue transglutaminase type 2 (tTG), endomysial antibodies (EMA) and antibodies against deaminated forms of gliadin peptides (DGP), while simultaneously testing for immunoglobulin A deficiency. If patients are IgA deficient, additional test measuring IgG class CD antibodies are advised. Duodenal biopsy histological features are graded using the Marsh-Oberhuber classification. Characteristically, there is villous atrophy with crypt hyperplasia and increased intra-epithelial lymphocytes in coeliac disease. Positive serology (tTG or DGP) in the presence of Marsh grade 2 or 3 duodenitis confirms the diagnosis of CD. (1)

CD screening is however not universally practised in all diabetic clinics in South Africa. In 2016, Tayob et al. described CD serological screening results in 120 newly diagnosed T1DM patients in Durban, South Africa. The serology test used in study included anti gliadin antibodies (AG), EMA and tTG. A limitation of this study is the use of an outdated antibody test (AG) leading to a very high serology positive group (40.8%).(23). AG serology is often positive in variety of other gastrointestinal disorders leading to a high false positive result for CD (4). A small number of serology positive patients underwent biopsies (16%) and

confirmed the presence of CD in three patients (2.5%) of the total population. The study lacked a description of the ethnicity of the confirmed CD patients.(23)

South Africa is the largest recipient of migrants from the Southern African Development Community.(24) Global geographic distribution of CD are influenced by changes in wheat consumption and migratory flow patterns.(3) The population of the clinic in this study consists of a variety of race groups and a large migrant population.

This study examines the prevalence of biopsy confirmed CD in a T1DM paediatric clinic in Johannesburg, South Africa. It describes the demographics and symptoms of CD confirmed patients and compares findings to other international studies.

What is already known on this topic?

- CD is a well-known co-morbidity in T1DM patients
- CD prevalence may be underestimated in developing world
- Untreated CD is associated with significant morbidity

What this study adds:

- Data of CD prevalence in a multi-ethnic paediatric T1DM population in Johannesburg
- Highlights the importance of migration on prevalence of CD in South Africa
- Confirms the importance of routine screening programs for CD in T1DM clinics

Methods:

Setting and participants:

A retrospective audit was done at a single tertiary academic hospital in Johannesburg, South Africa. Inclusion criteria were all type 1 diabetic patients following up at the outpatient paediatric endocrine clinic between January 2014 and March 2017. Patients with incomplete records and where CD screening was omitted or incomplete were excluded.

Data Collection:

Anonymised data collected from T1DM files included gender, age, age of diagnosis of T1DM, ethnicity, weight, height, CD serology results, biopsy results with Marsh grading, total IgA values and symptoms in biopsy confirmed CD patients. Questioning on symptoms were further explored by treating clinician if serology and histology were positive.

Growth was assessed using WHO Z-score values for height, BMI for age for children over five and weight for age for children under five. Weight and height values were converted to z scores using WHO AnthroPlus software.(25) Patients were assessed based on their BMI for age z-scores as normal (> -2), thin (between -2 and -3) or severely thin (<-3)in children over five years of age. Children under five years were assessed by z-scores for weight for height as wasted (<-2) or severely wasted (<-3). Height for age z-scores were defined as normal (>-2), stunted (between -2 and -3) or severely stunted (<-3).

The serology tests done in all patients included IgA and IgG titres of DGP and tTG. All patients with any positive serology results as per National Health Laboratory Services were

offered an upper endoscopy with duodenal biopsies, and a diagnosis of CD was made in patients with a histological pattern of Marsh two or three lesions as per ESPGHAN guidelines.(1) Biopsies were standardized by use of a single endoscopist with a standard number and site of biopsies as described by the ESPGHAN diagnostic guidelines (1).

Ethnical background information was collected from files and patients were grouped as South African Black, Coloured (South African descent, mixed race), White, Indian or African immigrant /originating from Africa but not Southern Africa.

Study data collected were managed using REDCap electronic data capture tools hosted at the University of Witwatersrand.(26) Data analysis was done using Stata Statistical software, release 11 (Statacorp, USA).

Ethical clearance was obtained from the Human Research Ethical Committee of the University of the Witwatersrand (protocol number: M170237)

Statistical Methods:

Continuous data was tested for normality and presented using means and standard deviations or medians and interquartile ranges as appropriate. Mann Whitney U tests were performed to compare non-parametric continuous data and Chi squared tests with Fisher exact statistics (where appropriate) were used to compare categorical data.

Results:

General Characteristics

89 patients' records were retrieved, of which 13 were excluded from the study. Two patients had type 2 diabetes and 11 patient's records were incomplete in the exclusion group. Half of the remaining 76 patients were male (38, 50%). The median age was 142.5 months (range 23-200). Ethnic backgrounds of patients were South African Black patients (25, 32.9%), South African Coloured (22, 28.9%), South African White (12, 15.8%), South African Indian (5, 6.6%) and African immigrant (12, 15.8%).

CD Screening results

Eleven of the participants had positive serology results for CD. All patients with CD positive serology were offered duodenal biopsies which two patients declined. The two patients that declined biopsy included a 14 year old white South African male who tested positive with high titres for all four serology tests (IgA tTG 600U/mL, IgG tTG 45U/mL, IgA DGP 38U/mL, IgG DGP 37U/mL. The second patient was an 8 year old black South African female who only tested positive for IgG DGP (titre 23U/mL). Both patients were asymptomatic and thriving.

Of the nine duodenal biopsies, six patients (6/74, 8%) had Marsh grading scores of two or three confirming CD. Of the three negative biopsies, all scored as Marsh 0, one had only positive tTG IgA (titre 1:44), and two had only a positive DGP IgA (titres 1:43 and 1:46). (Figure 1)

IgA

Total IgA levels were included in initial CD screening of all T1DM. The result was however not consistently available. Three of 61 (4.9%) patients had low total IgA levels but tested negative for CD with serology IgG tests for tTG and DGP. They were all thriving and asymptomatic.

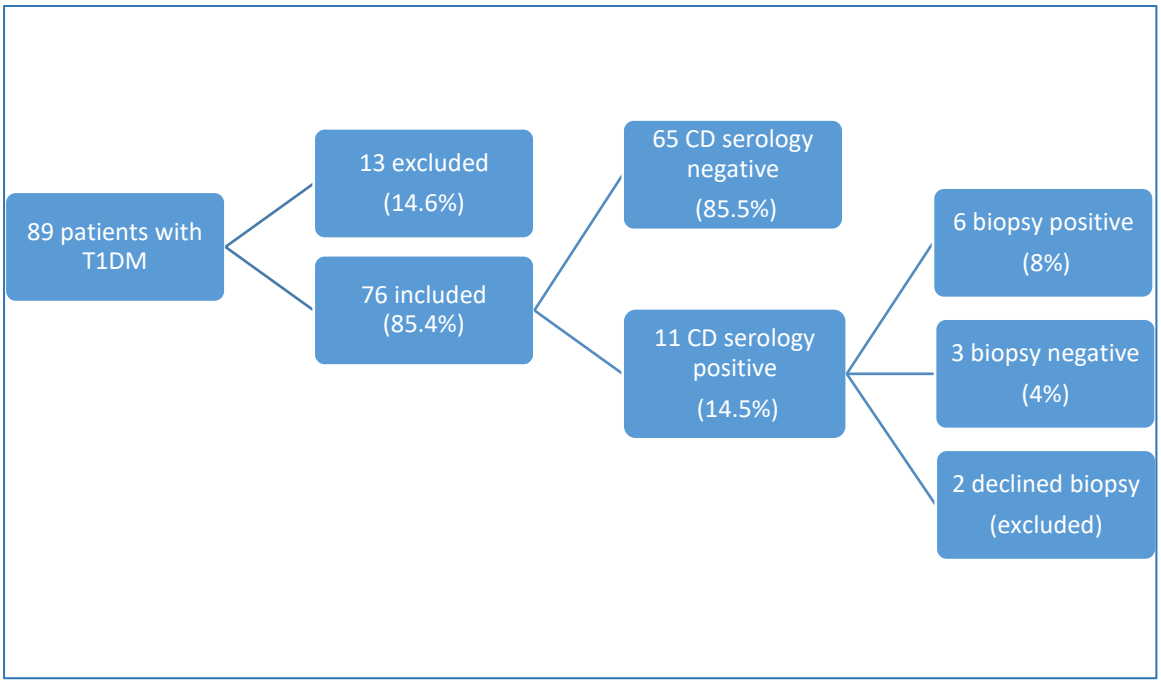

Figure 1. Study flow chart

Table 1. Coeliac patients

Age (months)	Ethnicity	Gender	Age at diagnosis of T1DM (Months)	Age at diagnosis of CD (Months)	Antibodies positive	Marsh grade biopsy result	Height for age z-score	BMI for age z-score	Symptoms*
61	African immigrant	Male	42	49	DGP IgA&G tTG IgA&G	2	+0.88	+0.33	Dental caries
171	SA White	Female	120	155	DGP IgA&G tTG IgA&G	3c	-0.39	-0.13	Fatigue, poor appetite
155	African immigrant	Female	36	149	DGP IgG tTG IgA&G	2	-2.12	+0.72	Fatigue, dermatitis herpetiformis
115	African immigrant	Male	96	106	DGP IgG tTG IgA&G	3b	-0.28	-0.55	Constipation
151	SA Indian	Male	72	135	DGP IgG tTG IgA&G	3c	+0.99	+0.29	None
140	SA White	Male	132	133	DGP IgA&G tTG IgA&G	3c	-1.28	+0.11	Abdominal pain

tTG, tissue transglutaminase type 2; DGP, deaminated forms of gliadin peptides

*CD symptoms were only identified after obtaining a positive screening result for CD

Confirmed Coeliac Disease group (Table 2)

General

There were four males (67%) and two females (33%) in the biopsy proven CD group. There was no difference in the age at diagnosis of T1DM between the CD and non-CD patients (Median age 84 vs 72 months, p= 0.64).

Anthropometry

In the CD group, anthropometric data was normal for all, except one patient who was stunted. In the non-CD group twelve patients were stunted (15.8%) and five patients were thin (6.6%).

Ethnicity

The migrant population attending the T1DM clinic are descendants from multiple African countries. There were no South African Black or Coloured patients in the CD group. There was one South African Indian, two South African White patients, and three (50%) migrant patients from other African Countries (Somalia and Ethiopia) in the CD group. Of note is that one patient who declined biopsy was a white South African male patient that had IgA tTG titres more than ten times upper normal limit, which has a high likelihood of being associated with a Marsh grading of three according to ESPGHAN guidelines(1)

Symptoms

Patients were screened for symptoms of CD at diagnosis of T1DM and again on finding positive serology and histology results for CD. Classical gastrointestinal symptoms were notably lacking in CD group, with only three patients reporting a single gastrointestinal symptom each. One patient had dental caries and one dermatitis herpetiformis. Fatigue was the most common reported complaint (33.3%).

Comparative analysis (Table 2)

CD was more common in South African White, South African Indian, and African immigrant patients, but only the comparison between African immigrants and all other patients reached statistical significance (p= 0.04).

Table 2. Characteristics of coeliac disease and non- coeliac disease patients

	Coeliac disease N= 6	**Non-coeliac group N= 68**	**p-value * **
Age at diagnosis of T1DM (months): Median (IQR)	84 (42, 120)	72 (48, 108)	0.64
Gender – Male n (%) - **Female n (%)**	4 (67%) 2 (33%)	33 (49%) 35 (51%)	0.34
Height for age z-score Median, (IQR)	-0.34 (-1.28, 0.88)	-0.55 (-1.5, 0.27)	0.67
Stunted (HAZ < -2) n (%)	1 (17%)	12 (18%)	0.72
BMI for age z-score Median (IQR)	0.2 (-0.13, 0.33)	-0.09 (-1.05, 0.63)	0.46
Thin (BMI for age z-score <-2) n (%)	0 (0%)	5 (7%)	0.64
Ethnicity: n(%) - **SA Black** - **SA Coloured** - **SA Indian** - **SA White** - **African immigrant**	 0 (0%) 0 (0%) 1 (17%) 2 (33%) 3 (50%)	 24 (35%) 22 (32%) 4 (6%) 9 (13%) 9 (13%)	 0.09 0.17 0.35 0.22 0.04
Serology: n (%) - **TTG IgA positive** - **TTG IgG positive** - **DGP IgA positive** - **DGP IgG positive**	 6 (100%) 6 (100%) 4 (67%) 6 (100%)	 1 (1.5%) 0 (0%) 2 (3%) 0 (0%)	 <0.001 <0.001 <0.001 <0.001

* p-value comparing coeliac versus non-coeliac groups

Discussion

This study demonstrated a prevalence of 8% of biopsy confirmed CD in a South African T1DM clinic. This finding is similar to that of other international studies. It is an important finding due to lack of consistent CD screening programs in South African Diabetic clinics. In this study 50% of confirmed CD patients were African immigrants and 33% South African White patients with a European heritage. Immigrant status was a statistically significant risk factor for having CD (p= 0.04) An important negative was that the study showed no South African Black or Coloured patients with CD in a clinic that was largely made up of South African Black (33%) and Coloured (29%) patients. However, this study was not powered to

prove a lack of CD in the South African Black population, and a larger study sample size will be needed to confirm or refute this.

There are several possible explanations other than the small sample size that may explain the absence of CD in the South African black population. Firstly, the prevalence of HLA DQ2 and DQ8 in our population is unknown and may be low. Secondly, gluten intake is likely to be lower in a predominantly maize-based diet fed population. Lastly, the sensitivity of coeliac serology has not been tested in the South African black population.

The findings among the confirmed CD group highlighted that CD cannot be excluded based on normal growth parameters and lack of gastrointestinal symptoms. Height-for-age and BMI-for-age were mostly normal among CD patients and no patients presented with gastrointestinal complaints. Only on specific questioning after serology and histology results were known to treating clinicians did three out of six patients report a single gastrointestinal symptom which included abdominal pain, poor appetite and constipation. This confirms previous findings that CD is often silent and asymptomatic. The aim is to intervene by introducing a gluten-free diet (GFD). Prompt diagnosis and management and GFD adherence can reduce complications seen in CD combined with T1DM. GFD adherence improves inflammatory immune response, improves BMD and has the potential to reduce urine albumin excretion. There is also the benefit of preventing long term complications such as cognitive impairment related to iron deficiency, malignancy and retinopathy. (1,4,6,9,21,26–29)

CD prevalence is higher in patients with total IgA deficiency. Immunoglobulin A antibodies against tTG and DGP may be falsely negative in these patients. In this study group, three of the patients (4.9%) had low total IgA levels, which is similar to international studies with a prevalence of 5.3-5.9%(30,31). All three of these patients were thriving, asymptomatic with

negative IgG serology results. Further annual screening with IgG serology is recommended.(1)

Study Limitations

Limitations in this study includes a small sample size of patients, and its retrospective nature meant the presence or absence of symptoms could not be confirmed in the non-coeliac population. 2/11 (19%) of patients with positive serology refused endoscopy and biopsy, thus could not confirm CD. Lastly, inherent to serology based screening programmes, the presence of sero-negative coeliac disease cannot be excluded.

Conclusion

To the best of the author's knowledge this is the first estimate of prevalence of CD in an at-risk population from South Africa utilising newer sensitive serology and biopsies according to ESPGHAN and ISPAD guidelines. Different practices with regards to screening for CD in T1DM patients exist, and the study highlights the need to screen all T1DM patients on a regular basis, regardless of growth parameters, presence or absence of symptoms, or racial group.

The absence of South African Black/Coloured patients with CD in this study does not confirm the absence of CD in this population group, and further studies with larger sample sizes are required to assist with local recommendations on screening frequency and timing. Currently there is no data in South Africa available on HLA typing of CD patients and future studies could be helpful to show which individuals are genetically susceptible to CD in our population.

Acknowledgements

We thank the superintendent of our study hospital for their permission to carry out this research.

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Appendix 1

Research Protocol:

Title: Coeliac Disease in a South African paediatric type 1 diabetes mellitus clinic.

Investigator: Carla Basson, MBChB (Medunsa), BSc (Stellenbosch)

Student no: 0311281Y

Degree: MMed (Paed)

Supervisors: Tim De Maayer, MBBCh (Wits), FCPaed (SA), MMed (Paed), Cert Gastronterol(SA) Paed

Dr Nicole van Wyk, MBBCh (Wits), DCH (SA), FCPaed (SA)

1. Introduction – Background Information

Coeliac disease (CD) is an autoimmune mediated systemic condition that is triggered in genetically susceptible individuals by diets containing gluten and related prolamines.(1) (32). It is characterized by a variety of gluten-dependent gastrointestinal and non-gastrointestinal signs and symptoms, enteropathy, human leukocyte antigen HLA-DQ2 and/or HLA-DQ8 haplotypes and CD specific antibodies.(4,8,32)

Gluten is a combination of proteins (prolamines) that are found in the endosperm of wheat called gliadins and glutenins, hordeins found in barley and secalins in rye. Gliadins are considered the most immunogenic.(22,32)

The prevalence of CD is approximately one to three percent in most European descent populations(32). It also occurs in populations of northern Indian, West Asian and South Asian descent. (22) Only sporadic data exist from Africa and Latin America, but studies done to estimate the burden of CD in these populations have postulated it to be an underdiagnosed and neglected component of diarrhoeal diseases globally.(33)

Gastrointestinal or typical symptoms include recurrent abdominal pain, abdominal distention, diarrhoea, nausea, vomiting, chronic constipation, flatulence and anorexia.
Nongastrointestinal or atypical symptoms include failure to thrive, short stature, dermatitis herpetiformis, recurrent aphthous stomatitis, delayed puberty, dental enamel defects, rickets,

osteoporosis, iron deficiency anaemia unresponsive to treatment, fatigue and abnormal liver function. (8)

Long term complications of untreated CD include failure to thrive, delayed puberty, poor dental enamel, osteopenia, osteoporosis, bone fractures and infertility. There is also an increased risk of developing intestinal malignancy, specifically Hodgkin's/ non-Hodgkin's lymphoma and small bowel carcinoma.(8)

Children can present with the typical gastrointestinal manifestations, nongastrointestinal manifestations or be asymptomatic at diagnosis. The latter group is usually identified through screening of high risk groups.(8)

CD has a strong genetic component. First degree relatives of CD affected individuals have a five to 30 percent chance of being affected, with a significant increase in twins. (22) There is a strong association between CD and certain autoimmune disorders.(32)

The 2012 ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) guidelines consist of an evidence-based approach to diagnosing CD. The guidelines advise testing should be offered to two groups. The first group includes children and adolescents who present with unexplained typical or atypical symptoms. The second group includes children and adolescents with an increased risk of having CD, regardless of whether they are symptomatic or not. This group includes patients with Type 1 Diabetes Mellitus, Down Syndrome, Autoimmune Thyroid Disease, Turner Syndrome, Williams

Syndrome, Selective Immunoglobin A Deficiency, Autoimmune Liver Disease and first degree relatives with CD. (1)

Diagnostic tools include CD specific antibody tests, HLA testing for HLA- DQ2\ DQ8 and histological analysis of duodenal biopsies. The specific antibodies include tissue transglutaminase type 2 IgA antibodies (TG2), endomysial IgA antibodies (EMA) and IgA antibodies against deaminated forms of gliadin peptides (DGP). Total IgA should also be done and if individual is IgA deficient, IgG levels should be tested for the CD specific CD antibodies.(1) Available serology test are highly sensitive and specific for CD. IgA-EMA and IgA-TG2 sensitivity and specificity are more than 90 percent and IgA-DGP sensitivity and specificity are more than 80 present.(34)

HLA testing is useful to exclude CD.(1) 95 percent of CD patients have HLA-DQ2 and five to ten percent carry HLA-DQ8. (8) If an individual is negative for both alleles, the diagnosis of CD is unlikely. (22)

Duodenal biopsies done with upper endoscopy should include at least one specimen from the duodenal bulb and at least four specimens from the second and third portion of duodenum. The classical histological features include villous atrophy with crypt hyperplasia and intra-epithelial lymphocytes.(8). Histology is graded using the Marsh-Oberhuber classification.(1)

As mentioned Coeliac disease is a well-known co-morbidity of type 1 Diabetes.(7) Elfstrom, Sundstrom and Ludvigsson article ‘Systematic review with meta-analysis: associations

between coeliac disease and type 1 diabetes' found a prevalence of biopsy confirmed CD to be six percent in type 1 diabetics.(21). Other studies report a mean prevalence of four and a half percent with the majority advocating for routine screening of Type 1 Diabetics for CD(2,5–7,21,35,36).

Many diabetics are asymptomatic for CD at diagnosis, but studies have found a significant difference in height and weight standard deviations and decreased bone mineral density attributable to delayed diagnosis of CD. (5–7,36). CD is also thought to have an adverse effect on glycaemic control in Type 1 diabetic.(5)

Recent epidemiological data show that CD is prevalent in the world, affecting not only people of European ancestry, but also populations of the developing countries(3,37). In Northern Africa there are studies that report high incidences of CD in their general population and in at risk groups. A study in West Algeria found the highest reported frequency of CD in Insulin dependent Diabetes Mellitus in the world of six percent.(38) There is a significant lack of data on CD in South Africa. On preliminary searches there was only one study specific to South Africa from 1981 reporting on twenty adult patients with Coeliac disease.(39). The author found no data on CD prevalence in the South African Type 1 diabetic population.

At Rahima Moosa Mother and Child Hospital all Type 1 diabetics are routinely screened for CD. Tests include serology (TG2, DGP) and total IgA levels. All patients that have positive serology results undergo duodenal biopsies. This approach follows ESPGHAN guidelines.(1) In the public sector genetic testing is not routinely offered.

2. **Problem Statement**

Coeliac disease is a significant comorbidity of Type 1 Diabetes. There is minimal data available on the prevalence in the South African health care setting. This study proposal aims to look at the diabetic population at Rahima Moosa Mother and Child hospital CD serology screening and biopsy results.

3. **Study Aim & Objectives**

a. **Aim**

To determine the prevalence of positive CD serology and duodenal biopsy results in the Type 1 Diabetic, Paediatric population presenting to Rahima Moosa Mother and Child Hospital since January 2014.

b. **Objectives**

i. Determine the percentage of children presenting with positive serology for CD in Type 1 Diabetic patients.
ii. Determine percentage of patients with biopsy confirmed CD.
iii. Describe the demographics of children with positive serology

iv. Describe prevalence of CD symptoms in patients that test positive for CD in Type 1 Diabetic group.

4. Methods

a. Study Design

A retrospective audit of the records of Type 1 DM children who were seen and screened for CD at Rahima Moosa Mother and Child hospital from January 2014 until March 2017.

b. Sample Population

i. Inclusion Criteria

All Type 1 Diabetic patients following up at Rahima Moosa Endocrine clinic between January 2014 and March 2017.

ii. Exclusion Criteria

Patients with incomplete information or where CD testing was omitted.

c. **Procedures**

Data will be collected from patient records kept by the Paediatric Endocrinology department at Rahima Moosa Mother and Child Hospital. Permission will be obtained from the hospital authorities and the Department of Paediatrics at Rahima Moosa Mother and Child.

d. **Data Handling, collection and analysis.**

The researcher will manually go through the files of all Type 1 DM patients and enter collected data on data capture forms. Study data collected will be managed using REDCap electronic data capture tools hosted at the University of Witwatersrand. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies.(26). Data analysis will be done using Stata software.(40)

All blood results will be retrieved from the National Health Laboratory Trackcare system. A statistician will be consulted to verify statistical analysis. Growth parameters will be converted to z-scores using the WHO growth standards and the WHO Anthro programme.(25) Continuous parameters such as anthropometric measurements and age will be assessed for normality of their distribution and described using means and standard deviations or medians and interquartile ranges as appropriate. Differences between those with CD and those without will be tested using the Student t-test or Kruskall-

Wallis test as appropriate. Categorical data such as genetic background (race and country of origin) will be assessed using the Chi square test and likelihood ratios. A p value below 0.05 will be considered significant. No sample size calculation was performed as it is the researcher's intention to include all patients that have been tested for CD in this clinic.

5. **Significance**

There is a global interest in CD and its association with other auto immune diseases. Limited studies are available on CD screening programs in at risk groups in South Africa, and Africa as a continent. This study will attempt to elicit the prevalence of CD in our Type 1 Diabetic population and compare our findings to other international studies. Our findings could be used to highlight the need to screen all asymptomatic type 1 diabetics for CD to prevent complications associated with undiagnosed CD.

6. **Limitations**

- The study is a retrospective audit of records and thus relies on data from existing records. These records may not be complete and may weaken the study.
- The clinic is run by a limited number of doctors and files are kept in the clinic to minimize incomplete records and lost files.
- Our population group is a small sample size of 90 patients.

7. Ethical Considerations

The proposal for this study will be submitted to the Postgraduate Committee of the University of the Witwatersrand and it will be submitted to the Human Research Ethical Committee (HREC) of the University of the Witwatersrand for approval. As this is a retrospective audit, no consent will need to be obtained from individual patients. Data sheets will only contain a study number. The patient's name and file numbers will be stored in a separate database at a different, secure location so that only the investigator and the supervisor will have access to this information.

8. Timeline

	Nov 2016	Dec 2016	Jan 2017	Feb 2017	March 2017	April 2017	May 2017	Jun 2017
literature review								
protocol preparation								
protocol assessment								
ethics approval								
post graduate approval								
data collection								
data analysis								
write up report								
write up paper								

9. Cost/Funding

The cost involved in the study is for stationery, printing and binding. This cost will be borne by the investigator as there is no funding for this study.

10. **Resources**

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Data capture sheet

Study number: _____

Age at diagnosis of DM (months) _____

Ethnicity: _____

Citizenship: _____

Gender: Male Female

Weight (kg): _____ Weight for age z-score: _____

Height (cm): _____ Height for age z-score: _____

Weight for height z-score: _____

Results:

Anti TTG IgA: Positive Negative Titre if positive: _____

Anti TTG IgG: Positive Negative Titre if positive: _____

Anti DGP IgA: Positive Negative Titre if positive: _____

Anti DGP IgG: Positive Negative Titre if positive: _____

Biopsy done: Yes No N/A

Marsh score: _____ or Reason not done: _____

Symptoms (Collect for CD patients only)**:**

Diarrhoea ☐ Vomiting ☐ Nausea ☐

Constipation ☐ Abdominal pain ☐ Fatigue ☐

Fractures ☐ Ferritin level if taken: _____

Appendix 2

R14/49 Dr Carla Basson

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170237

NAME: (Principal Investigator)	Dr Carla Basson
DEPARTMENT:	Paediatrics Rahima Moosa Mother and Child Hospital Paediatric Endocrine Clinic
PROJECT TITLE:	Coeliac Disease in a South African Paediatric Type 1 Diabetes Mellitus Clinic.
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Tim De Maayer and Nicole van Wyk
APPROVED BY:	Professor P. Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	29/03/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially review in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3

Turnitin Originality Report

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Appendix 4

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

CERTIFICATE OF SUBMISSION FOR EXAMINATION OF MASTERS RESEARCH REPORT / DISSERTATION OR PHD THESIS SIGNED BY HIGHER DEGREES CANDIDATES

Full name	Carla Basson		
Student number	0311281Y		
Title of submitted Research Project: Coeliac Disease in a South African Paediatric Type 1 Diabetes Mellitus clinic.			
Contact no	082 564 9287	E-mail	carlabasson@gmail.com

1. If you are likely to move in the next 6-12 months, please give the anticipated date of move: N/A
2. I hereby submit my **Masters (research report) / Masters (dissertation) / PhD thesis for examination** (Select whichever is applicable)
3. I have checked all copies of my research report and declare that no pages are missing or poorly reproduced.
4. I have submitted 2 bound copies and 1 copies on CD

5. **I confirm that I have:**
   a) A signed declaration indicating my understanding of the concept of plagiarism and a denial of plagiarism in my research document.
   b) A report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document included as an appendix.

6. **I confirm that I have:**
   a) Not used either human or animal tissue or records **No**
   b) If yes: I have included the ethics waiver letter pertinent to my research as an appendix **N/A**
   c) Done research using animals **No**
      If yes: I have included a copy of the animal ethics committee clearance certificate as an appendix in this document **N/A**
   d) Done research using human subjects, human tissue or patient records **Yes**
      If yes: I have included a copy of the human ethics clearance certificate as an appendix to the research document **Yes**

7. I understand that I may not graduate unless my University fees have been paid in full.
8. My Supervisor(s) names, departments, telephone numbers and email addresses are as follows:

Name	Dr Tim De Maayer		
Department	Rahima Moosa department of Paediatrics and Child Health		
Telephone	083 424 1607	E-mail	Tim.DeMaayer@wits.ac.za
Name	Dr Nicole Van Wyk		
Department	Rahima Moosa department of Paediatrics and Child Health		
Telephone	082 562 1554	E-mail	Nicole.VanWyk@wits.ac.za

List all publications, which you have published in peer-reviewed journals from your postgraduate research report/dissertation/thesis during the course of your studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory. **None**

Signature of candidate: Basson Date: 14/07/19

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Appendix 5

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

CERTIFICATE OF SUBMISSION FOR EXAMINATION SIGNED BY SUPERVISORS OF HIGHER DEGREES CANDIDATES

Full name	Carla Basson		
Student number	0311281Y		
Candidate for the degree of: Master of Medicine in Paediatrics *has submitted his/her thesis/dissertation/research report* Entitled: Coeliac Disease in a South African Paediatric Type 1 Diabetes Mellitus clinic			
Contact no	0825649287	E-mail	carlabasson@gmail.com

Mark with an X on appropriate box	Yes	No
Has this thesis/dissertation/research report been submitted with the acquiescence of the supervisor?	X	
To the best of your knowledge are you able to verify that this is the candidate's work, except as otherwise stated by the candidate?	X	
The substance (nor any part of it) has not been submitted in the past nor is being submitted for a degree in any other university?	X	
The candidate has acknowledged wherever any information used in the thesis, dissertation or other work has been obtained by him/her while employed by, or working under the aegis of, any person or organization other than the University or its associated institutions?	X	
Have examiners been nominated and approved?	X	

I certify that this thesis/dissertation/research report has the approval of the Animal Ethics Committee / Committee for Research on Human Subjects and the Number of the Certificate of Approval is:

M170237

List all publications, which your student has published in peer-reviewed journals from his/her postgraduate research report/dissertation/thesis during the course of his/her studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory.
None

Name of Supervisor 1: Nicole Van Wyk

Telephone: 0825621554 Email: Nicole.vanwyk@wits.ac.za

Signature: _____ Date: 14 June 2019

Name of Supervisor 2: Tim De Maayer

Telephone: 0834241607 Email: tim.demaayer@wits.ac.za

Signature: _____ Date: 14 June 2019

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