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Seroprevalence of SARS-CoV-2 IgG and IgM in Diabetic and Oncology patients at Pietersburg Hospital, Limpopo, South Africa

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ABSTRACT

Introduction: The global COVID-19 pandemic has disproportionately impacted people with weakened immune systems. Non-communicable diseases, which include diabetes and cancer, are the top causes of weakened immune systems, recording up to 74% of all deaths globally. Studying the seroprevalence is crucial in understanding the epidemiology of the virus and contributes to the improved management of COVID-19 amongst patients with cancer and diabetes. **Methods:** The study was a single-center prospective study that tested serum samples for routine chemical analysis from March to July 2022 at NHLS, Chemical Pathology, Polokwane laboratory using the COVID-19 IgG/IgM Rapid Test Cassette - Orient Gene Biotech, Zhejiang, China. The assay tests antibodies against SARS-CoV-2 in the early (lgM) and later stage (lgG) of infection. **Results:** Of the 207 patients with diabetes, 84% had detectable IgG and IgM antibodies against SARS-CoV-2. Similarly, 81% of the 283 cancer patients had detectable IgG and IgM antibodies against SARS-CoV-2. The patients with diabetes had a median age of 56 years (range: 0-91), and 60% were females. The cancer patients had a median age of 62 years (range: 49-72), and 40% were females. **Conclusion**: The study demonstrates a high SARS-CoV-2 IgG and IgM antibody seroprevalence in diabetic and oncology patients at Pietersburg Hospital, Limpopo, South Africa. These findings emphasize the importance of ongoing serologic testing to track the pandemic, particularly among immunocompromised patients, and inform the development of effective public health strategies to mitigate COVID-19 transmission.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), the causative agent for the coronavirus disease 2019 (COVID-19), has ravaged the world for over two years since reported in 2019 [1]. As of December 2022, the virus has caused over 650 million infections and over 6.5 million deaths worldwide [2]. South Africa had the most significant number of infections in Africa, recording over 4 million cases and over 100,000 deaths [2].

The global COVID-19 pandemic has disproportionately impacted patients who are immunocompromised. Non-communicable diseases (NCDs) cause up to 41 million deaths annually, translating to 74% of all deaths globally [3]. The World health organization (WHO) has identified cancer, chronic respiratory diseases, cardiovascular diseases, and diabetes as the four main types of NCD. South Africa, which is at an upward trajectory of NCDs, will soon be one of the "Big 5" homes of NCDs. In 2020, the prevalence of cancer was 16%, and diabetes was 7% among the general population in South Africa, two to three times higher than in developed countries [4, 5]. Studies have shown that cancer and diabetes patients with SARS-CoV-2 infection are at a 3.5 times higher risk of requiring mechanical ventilation or intensive care unit (ICU) admission than the general population [6]. Thus, investigating the dynamics of COVID-19 and cancer and diabetes is paramount.

Cancer patients are more susceptible to infections due to coexisting chronic diseases, poor health status, and systemic immunosuppressive states caused by cancer and anticancer treatments [7–9]. Diabetes causes immune response dysfunction; thus, diabetic patients are also at a higher risk. It is essential to understand the dynamics of COVID-19 disease in patients with diabetes and cancer to assess the risk of nosocomial transmission of COVID-19, considering the regular in- or outpatient visits.

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Seroprevalence surveys may provide a better understanding of the epidemiology of COVID-19 than the traditional molecular and antigen-based point-of-care assays due to their innate ability to detect past and recent infections in both symptomatic and asymptomatic infections. The antibodies against SARS-CoV-2 can be seen in the early (lgM) stages and later stages (lgG) of COVID-19 [10]. Seroprevalence studies also provide answers about patients' ability to develop antibodies [10]. Seroprevalence data on cancer patients have ranged from 3.6% in Australian studies to 30% in Spain and 67% in Germany [11–13]

South African studies of patients attending routine antenatal and HIV clinical care documented SARS-CoV-2 IgG antibody seroprevalence between 19% and 79.7% [14]. There is a paucity of seroprevalence studies on diabetes and cancer patients in South Africa. Thus, we sought to determine the prevalence of IgM and IgG COVID-19 seroprevalence survey in diabetes and oncology patients attending Pietersburg Hospital, Limpopo Province, South Africa.

MATERIAL AND METHODS

Ethical Approval. Ethics approval to conduct the study was issued from the Turfloop Research Ethics Committee of the University of Limpopo (TREC/496/2022: UG).

Data collection methods. We prospectively tested consecutive settled serum samples from diabetic and oncology patients referred for general routine checkups and outpatients scheduled for routine chemical analysis from March 2022 to July 2022 at the public referral NHLS, Chemical Pathology, Polokwane laboratory. All serum samples from diabetic and oncology patients were sent to the Chemical pathology laboratory for analysis. The demography, medical history, and type of cancer affecting the patients were recorded from the laboratory requesting forms submitted with the samples.

The serum was tested using the COVID-19 IgG/IgM Rapid Test Cassette, a lateral flow immune-chromatographic assay (Orient Gene Biotech, Zhejiang, China). The test cassette is a solid-phase immune-chromatographic assay used for the qualitative and differential detection of IgG and IgM antibodies to SARS-CoV-2 in serum or plasma. Test assay was performed according to the manufacturer's instruction within 72h of specimens received in the lab. Two trained technicians independently interpreted each result. When reading the assay, the technicians were blinded to the results of other assays and each other's results. A third technician arbitrated discordant reads between the two technicians. The test cassette contains colloidal gold conjugated to recombinant COVID-19 antigens (COVID-19 conjugates). When a specimen followed by assay buffer is added to the sample well, IgM and/or IgG antibodies, if present, bind to COVID-19 conjugates making antigen antibodies complexes. This complex migrates through the nitrocellulose membrane by capillary action. When the complex meets the line of the corresponding immobilized antibody (anti-human IgM and/or anti-human IgG), it forms a burgundy-colored band indicating a reactive test result. The absence of a colored band in the test region indicates a non-reactive test result. In this study, seroprevalence was the number of patients who tested positive for the IgG, IgM, or Both antibodies.

Statistical analysis. Data was recorded on MS Excel 2016 and later imported into SPSS v 27.0. Frequencies of IgG and IgM antibodies detected were generated, and seroprevalence was calculated. Stratified seroprevalence estimates were calculated for age group, gender, and cancer type. A chi-square test was used to compare the seroprevalence of males and females in diabetic and cancer patients and to compare IgG and IgM antibody frequencies by gender. A P-value ≤ 0.05 was considered significant.

Limitations of the study. This study had several limitations. The study was a single-center study conducted at Pietersburg Hospital in Limpopo Province. Thus, results are from a limited geographical area, potentially limiting generalizability to other countries. Secondly, information like the cancer stage at the time of collection was unavailable for cancer patients. At the same time, the type of diabetes was not specified. Lastly, vital information on co-morbidities and risk factors associated with COVID-19 infection, such as HIV status, obesity, and socio-economic status, were not obtained.

RESULTS

Of 3000 samples obtained from the Chemical pathology laboratory, 2374 were not classified as cancer or diabetic, and 136 were duplicates and thus excluded from the study (Fig 1). Thus, 490 samples from 207 diabetic patients and 283 cancer patients comprised our study sample.

Diabetic Patients

Demographics for diabetes patients. In the 207 diabetic patients, the median age was 56 years (range 0 – 91); 124 (60%) patients were women. Most patients, 48 (23.2%), were 55 to 64, followed by the age group 45 to 54 with 39 (18.8%). The least number of patients belonged to the age groups 05 to 14, with 2 (0.9%) and 00 to 04, with 4 (1.9%), respectively (Fig. 2).

Distribution of diabetic patients by seropositivity. The total seroprevalence of IgG was found to be 86%. A total of 154 (76%) patients were found to have IgG in their blood, while only 20 (10%) of them were found to have both IgG and IgM. Thirty-three (16%) patients were found to be negative at the time of testing (Fig 3).

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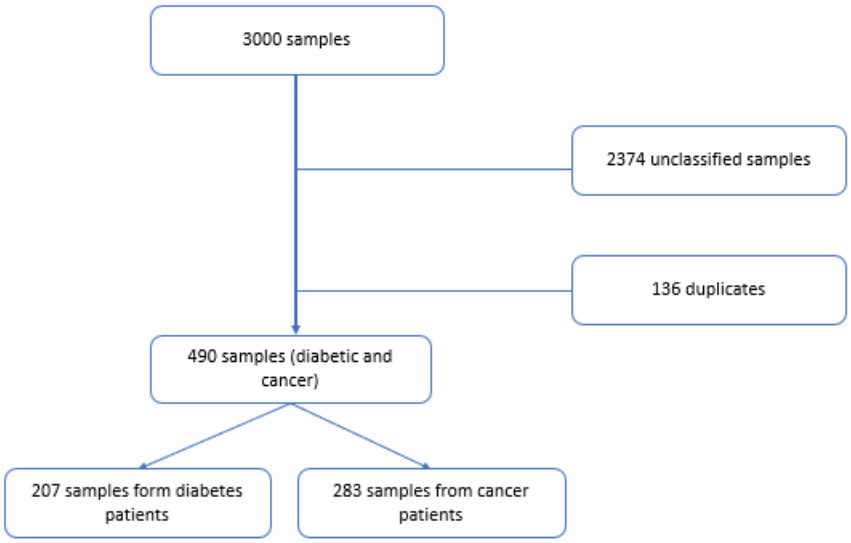


Fig. 1. Flow diagram illustrating the samples included in the study.

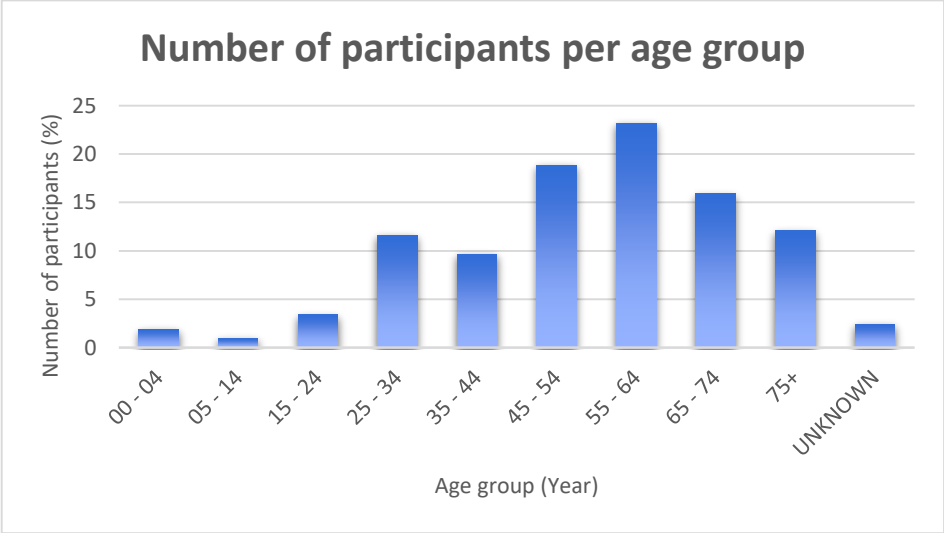


Fig. 2. Number of participants per age group among diabetes patients

Classification of diabetic patients by gender and antibodies. Overall, the seroprevalence of IgM and IgG was 60% in females and 40% in males. However, there was no significant association between gender and seropositivity, with *P* values of 0.562 and 0.993 for IgG and IgM (Table 1).

Distribution of diabetic patients by antibodies and age. Further classification of patients based on age and antibodies found that the age group 55-64 had the highest percentage of 25%, while the age group 00-04 had the lowest rate of IgG 0.57 %. The highest IgM seroprevalence of 25% was found in the age groups 45-54 and 65-74, while the lowest percent value of 10% was found in the age group 35-44. There was a significant association between age group and seropositivity, with *P* values of 0.001 and 0.033 for IgG and IgM (Table 2).

Cancer Patients

Demographic patterns of cancer patients. Of the 283 cancer patients, the median age was 62 years (range 49-72); 124 (60%) patients were women. The majority of patients, 66 (23.4%), belonged to the age group 65-74, followed by the age group 75+ with 57 (20%) patients. The least number of patients belonged to the age groups 00-04 and 05-1,4, with frequencies of 0.9% and 0.7%, respectively (Fig. 4).

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Fig. 3. IgG and IgM results from diabetic patients

Table 1. Association of SARS-CoV-2 antibodies and gender

Gender	N (%)	IgG Positives (%)	*P* value*	IgM Positives (%)	*P* value*	IgG + IgM Positives (%)	*P*-value*
Females	124 (59.90)	106 (60.92)	0.562	12 (60.00)	0.993	12 (60.00)	0.993
Males	83 (40.10)	68 (39.08)		8 (40.00)		8 (40.00)	
Total	**207 (100)**	**174 (100)**		**20 (100)**		**20 (100)**	

**P*-value was determined using Chi-square statistical test.

Table 2. Anti-SARS-CoV-2 IgG and IgM per age categories in diabetes patients

Age categories	N	IgG Positives (%)	*P*-value*	IgM Positives (%)	*P*-value*	IgG + IgM Positives (%)	*P*-value*
00-04	4	1 (0.57)		0 (0)		0 (0)	
05-14	2	2 (1.15)		0 (0)		0 (0)	
15-24	7	6 (3.45)		0 (0)		0 (0)	
25-34	24	18 (10.34)		0 (0)		0 (0)	
35-44	20	13 (7.47)		2 (10.00)		2 (10.00)	
45-54	39	31 (17.82)	<0.001	5 (25.00)	0.033	5 (25.00)	0.033
55-64	48	44 (25.29)		4 (20.00)		4 (20.00)	
65-74	33	30(17.24)		5 (25.00)		5(25.00)	
75+	25	24 (13.79)		4 (20.00)		4 (20.00)	
UNKNOWN	5	5 (2.87)		0 (0)		0 (0)	
Total	207	174 (100)		20 (100)		20 (100)	

**P*- value was determined using a chi-square statistical test.

Distribution of diabetic patients by seropositivity. The seroprevalence in cancer patients was 81%. Of the 283 cancer patients tested for IgG and/or IgM anti-SARS-CoV-2 antibodies, 212 (75%) patients were found to have the IgG antibody in their blood. Seventeen (6%) patients had both IgG and IgM, comprising 6% of the total population, and only 1 (0.3%) IgM positive result was recorded. Fifty-three (18%) patients were negative for IgG and IgM (Fig 5).

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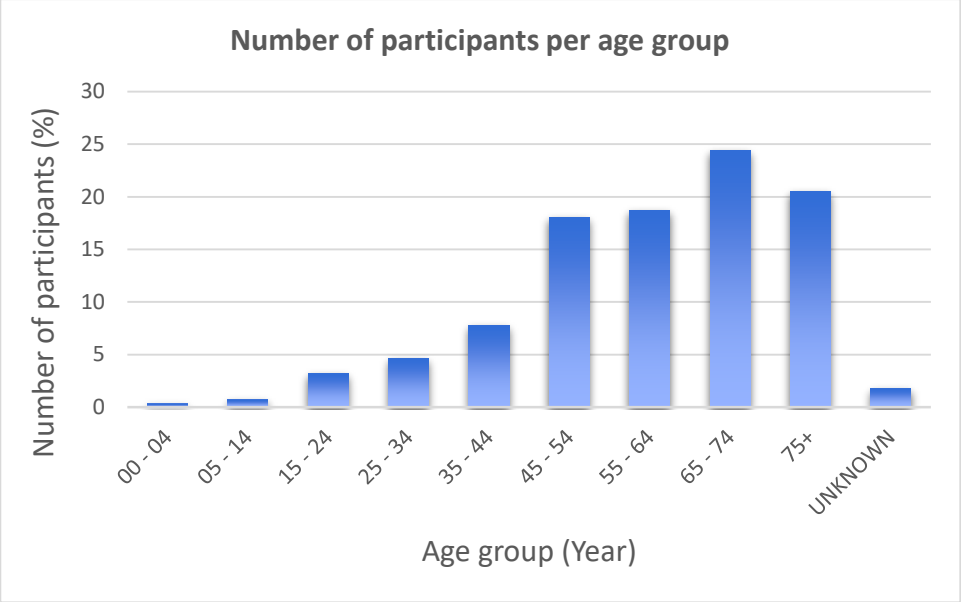


Fig. 4. Participants per age group among cancer patients.

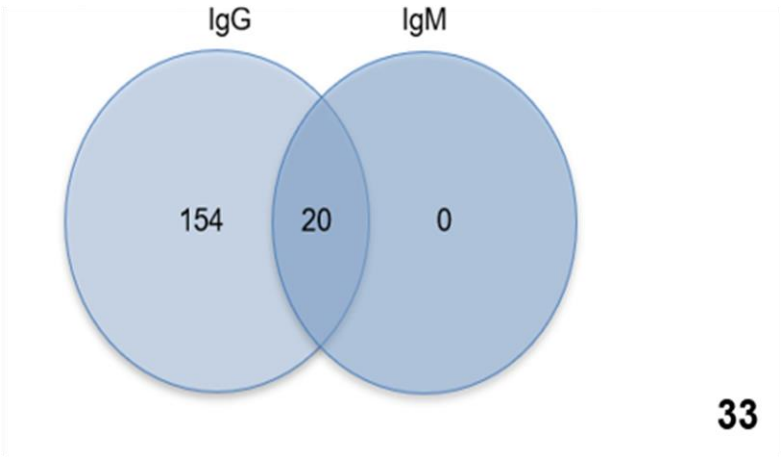


Fig. 5. Seroprevalence distribution of cancer patients

Distribution of diabetic patients by antibodies and gender. For IgG, males had a higher seroprevalence of 59% compared to 40% for females. For IgM, males also had a higher seroprevalence value of 67%, while females had 33%. However, there was no significant association between gender and seropositivity, with *P* values of 0.825 and 0.538 for IgG and IgM, respectively (Table 3).

Table 3. Anti-SARS-CoV-2 IgG and IgM per gender in cancer patients.

Gender	N (%)	IgG Positives (%)	*P*-value*	IgM Positives (%)	*P*-value	IgG+IgM Positives (%)	*P*-value*
Females	113 (39.9)	92 (40.2)	0.825	6 (33.3)	0.538	5 (29.4)	0.613
Males	168 (59.3)	135 (58.9)		12 (66.6)		12 (70.5)	
Unknown	2 (0.7)	2 (0.8)		0 (0)		0 (0)	
Total	**283**	**229 (100)**		**18 (100)**		**17 (100)**	

**P*-value was determined using the chi-square statistical test.

Distribution of diabetic patients' antibodies and age. Further, classification of antibodies by age found that the age group 65-74 had the highest number of patients, with 26% for IgG seroprevalence, while the age group 00-04 had the lowest percentage of IgG seroprevalence (0.44%). The highest IgM seroprevalence of 39% was found in the

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age group 65-74, while the lowest rate was in the age group 35-44 (5.55%). However, age and seropositivity had no significant correlation (Table 4).

Table 4. Anti-SARS-CoV-2 IgG and IgM per age categories in cancer patients

Age categories	N	IgG Positives (%)	*P*-value*	IgM Positives (%)	*P*-value*	IgG + IgM Positives (%)	*P*-value*
00-04	1	1 (0.4)		0 (0)		0 (0)	
05-14	2	2 (0.8)		0 (0)		0 (0)	
15-24	9	9 (3.3)		0 (0)		0 (0)	
25-34	13	12 (5.2)		0 (0)		0 (0)	
35-44	22	16 (6.9)	0.116	1 (5.5)	0.164	1 (5.8)	0.261
45-54	51	41 (17.9)		3 (16.6)		3 (17.6)	
55-64	53	41 (17.9)		2 (11.1)		2 (11.7)	
65-74	69	59 (25.7)		7 (38.8)		7 (41.1)	
75+	58	45 (19.6)		4 (22.2)		3 (17.6)	
UNKNOWN	5	3 (1.3)		1 (5.5)		1 (5.8)	
Total	**283**	**229(100)**		**18 (100)**		**17(100)**	

**P*- value was determined using the chi-square statistical test

Classification of cancer type and antibody distribution. The patients were further categorized according to the type of cancer. The most common cancer patients were prostate with 114 (40%), followed by breast cancer with 88 (31%). Patients who had prostate cancer had the highest number of IgG seroprevalence of 114 (50%), while the lowest IgG seroprevalence of 13 (6%) was found among patients with pancreatic cancer (Table 5).

Table 5. Distribution of antibodies and cancer type

Cancer type	N (%)	IgG Positives (%)	*P*-value	IgM Positives (%)	*P*-value	IgG +IgM Positives (%)	*P*-value
Prostate	114 (40)	114 (49.7)		11 (61.1)		11 (64.7)	
Breast	88 (31)	72 (31.4)	0.836	4 (22.2)	0.455	4 (23.5)	
Colorectal	35 (12)	30 (13.1)		1 (5.5)		1 (5.8)	
Pancreatic	16 (6)	13 (5.6)		2 (11.1)		1 (5.8)	0.652
Total	**283**	**229 (100)**		**18 (100)**		**17 (100)**	

**P*-value was determined using the chi-square statistical test.

Similarly, for IgM, the highest seroprevalence of 11 (61%) was found in patients with prostate cancer, and the lowest IgM seroprevalence of 1 (6%) was found in patients with colorectal cancer. However, the type of cancer and seroprevalence had no significant correlation (Table 5).

DISCUSSION

This study represents a single-center, prospective approach to investigate the seroprevalence of COVID-19 antibodies in outpatients and patients referred for general routine check-ups for cancer and diabetes treatment at Pietersburg Hospital, Limpopo.

In this study, the seroprevalence of IgG and IgM was 84% among patients with diabetes and 81% in cancer patients. This study showed a higher prevalence among diabetic and cancer outpatients and patients referred for general routine check-ups at Pietersburg Hospital, Limpopo Province.

In South Africa, pooled seroprevalence is estimated at around 60% in a rural community and 73% in an urban community among the general population [14, 15], while in Africa, pooled seroprevalence was lower, ranging from 0% to 63% [16]. However, in Europe, SARS-CoV-2 antibodies' seroprevalence studied among cancer patients found a prevalence of 31% for IgG or IgM at Bergamo Hospital, Italy [17]. Similarly, a study recorded a 31% COVID-19 IgM/IgG seroprevalence in Madrid, Spain [11]. In Austria, where the contagion was not so widespread, seroprevalence has been reported to be 1.4% between 21 March and 4 June 2022 [18]. In North India, 50.4% of patients with diabetes had SARS-CoV-2 antibodies [19].

Several reasons can explain the high prevalence of COVID-19 seroprevalence in this study. The current study was conducted in mid-2022, after the fourth wave in South Africa, due to the cumulative infections that occurred in the first to the fourth waves. Secondly, the current study included immunocompromised patients, who are more susceptible to COVID-19 disease. Evidence provided in a survey by Peykari et al. (2020) suggests that the estimated pooled prevalence of diabetes in people infected with COVID-19 as 7.87%, while the estimated pooled prevalence of cancer in COVID-19 patients is

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2.0%. The study further provides evidence that diabetic and cancer patients have a significantly increased risk of emerging severe COVID-19 compared to non-diabetic and non-cancer patients[20]. The higher prevalence reported in our study compared to the general population may also be due to the inclusion of patients with weakened immune systems due to diabetes and cancer. Diabetes increases mortality and morbidity risks during acute infections due to weakened humoral and innate immune functions [21]. Cancer patients are prone to infections due to coexisting chronic diseases, poor health status, and systemic immunosuppressive states caused by cancer and anticancer treatments [7–9]. However, the type of cancer had no significant bearing on the seropositivity results in this study.

In our study, most people with diabetes and cancer were older patients aged 45 and above. The higher infection in the older group may be due to patients with immunosenescence (weakening of immunity with age). Aging is characterized by a decrease in peripheral T cells of the blood, which may considerably increase susceptibility to COVID-19 [21, 22]. Similar trends in the severity of illness and mortality among adults ≥ 60 years were observed in China [24]. In Europe, mortality as high as 10% was in adults aged ≥ 79 years compared to <1% in young adults [25]. Another study in Iran reported that older cancer patients had severe outcomes for COVID-19[20].

When it comes to gender, the female group had a higher proportion of seropositivy compared to the males. In South Africa, women may be testing more than men since. Generally, they are better at seeking formal healthcare or testing and experience symptoms at a greater rate [26, 27]. However, the association of gender with seropositivity was not significant, implying it does not influence susceptibility to COVID-19. Similarly, Chitungo et al. (2020) found no significant association between seropositivity and gender in Poland [27]. Additionally, a study in Peru also found no significant variation in the seroprevalence of males (70%) and females (71%) [28]. Conversely, the findings were inconsistent with a study in Somalia, which suggested that the males had a higher risk of acquiring COVID-19 [29]. Nonetheless, the prevalence was inconsistent with the global pattern of male and female cases, showing no significant variation in confirmed cases [30]. Therefore, gender has no association with seropositivity and does not influence susceptibility to COVID-19 in Somalia. In both diabetes and cancer, there was no significant association between seroprevalence and both gender and age. The lack of significant association between gender and age and seropositivity (IgG and lgM) could indicate the uniformity of SARS-CoV-2 infection and did not discriminate with genders.

The high seroprevalence of more than 75% SARS-CoV-2 in this study for diabetes and cancer patients indicates widespread exposure to the SARS-CoV-2 and the possible presence of immunity against the virus. Reaching herd immunity, even among Pietersburg Hospital's most vulnerable patients with weakened immune systems, was good news. Cancer patients were overly cautious by avoiding gathering and attending their usual anti-cancer-directed therapy, which decreased the number of patients who received it. The seroconversion rate of our cohort, which was nearly the same for diabetes and cancer, is reassuring that most patients with cancer could mount an immune response to SARS-CoV-2.

Our results indicated that prostate cancer had the highest COVID-19 seroprevalence, while the lowest association was found for colorectal and pancreatic cancer. According to the study conducted in Mount Sinai, New York, the relationship between prostate cancer and COVID-19 was significantly influenced by the relationship between androgen and androgen receptor signaling. Androgen can enhance the expression of type II protease, TMPRSS2, a central molecule that mediates severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, by dampening the innate and adaptive immune responses [31]. However, the type of cancer had no significant bearing on the seropositivity results.

For both diabetes and cancer patients, IgM seropositivity was found to be relatively low, at 9% and 6%, respectively. IgG is detectable after one week and is kept at a high level for a considerable amount of time, whereas IgM is detected during the first week following SARS-CoV-2 infection [32]. IgM antibodies in this study indicate that the virus is still active in immunocompromised patients at Pietersburg Hospital and is spreading to new hosts. These findings warrant continued monitoring of COVID-19 seroprevalence in these patients, as in our study population, to limit the spread and severe outcomes of SARS-COV-2 disease in these patients.

In conclusion, the study showed a high COVID-19 seroprevalence among cancer and diabetes outpatients and patients referred for routine checkups at Pietersburg Hospital in Limpopo. The study also indicated the presence of recent infections besides the consensus that COVID-19 infection is dissipating. Thus, we must serologically monitor the antibodies in immunocompromised patients to prevent the spread and severe outcomes of this infection.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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