**Quantification and Categorization of Oral Candida among denture wearers with Type II Diabetes mellitus a****ssociated co-morbid conditions.**

**Abstract**

**Background:** Candida species are opportunistic pathogens that frequently colonize the oral cavity. Denture wearing and diabetes mellitus significantly enhance Candida growth and pathogenicity, potentially leading to severe infections. The interaction between these factors, especially in patients with comorbidities, requires further investigation.

**Objectives:** To evaluate Candida colonization quantitatively and categorize Candida species among type II diabetic complete denture wearers with co-morbid conditions.

**Methodology:** This cross-sectional observational study included 150 type II diabetic denture wearers with comorbidities. Saliva samples were collected, cultured on Sabouraud Dextrose Agar and CHROMagar, and incubated at 37°C for 24-48 hours. Colony-forming units (CFU) were counted, and species were identified based on colony morphology. Data were analyzed using chi square test, ANOVA and post-hoc Tukey tests, p-value<0.05 considered statistically significant.

**Results:** The study population was predominantly male (63.3%) and over 50 years of age (80%). Significant differences in Candida colonization were observed across comorbidity groups (p=0.036). Endocrine conditions demonstrated the highest mean Candida colonization (921.7±98.6 CFU), followed by cardiovascular (856.4±124.8 CFU) and gastrointestinal conditions (825.6±132.4 CFU). Post hoc analysis revealed significant differences between endocrine conditions and nephrological (p=0.031), and neurological conditions (p=0.004). Candida albicans was the predominant species (57.3%), with highest counts in endocrine conditions (608.3±65.1 CFU). Candida parapsilosis (18.8%), Candida tropicalis (11.9%), Candida glabrata (9.2%), and Candida krusei (2.0%) were also identified, with significant distinct distribution patterns across comorbidities.

**Conclusion:** Type II diabetic denture wearers with endocrine and cardiovascular comorbidities exhibit significantly higher Candida colonization, particularly C. albicans. These findings highlight the need for targeted oral health interventions and regular monitoring in this high-risk population to prevent oral fungal infections and potential systemic complications.

**Keywords:**  Candida colonization, Type II diabetes mellitus, Complete denture wearers, Oral candidiasis, Comorbid conditions

**Introduction**

Though fungi constitute a very small part of the oral microbiota, their role in oral and general health cannot be underestimated1. Candida species are the most frequent fungi to colonize the oral cavity and have adapted to reside as commensals2. The rate of colonization increases with age, systemic diseases immunity, illnesses such as cancers and intra-oral devices including dentures3. Candida albicans and Non-albicans Candida (NAC) species such as *Candida glabrata, Candida krusei,* and *Candida tropicalis* have been identified as important opportunistic pathogens and a cause of infection in oral cavity leading to discomfort and pain, an altered sense of taste, difficulty in eating and swallowing and consequently poor nutrition 4,5,6. Among immune-compromised patients, Candida infection can disseminate through the bloodstream causing severe infection with increased morbidity and mortality. The mortality rate from systemic candidiasis is up to 79% 7,8.

Denture wearing has shown to significantly enhance the growth and pathogenic activity of oral candida9. It causes specific conditions identified as Candida-associated denture stomatitis (CADS)10 and chronic erythematous candidiasis with prevalence of 60-65%11and 69.38%12 respectively.

Diabetes mellitus is a complex debilitating disease, that can affect most of organ system in the body, compromising the immune system and often fatal if left untreated13. Global prevalence of diabetes mellitus of around 382 million people in 2013, is projected to increase to 592 million by 203514. Diabetes mellitus due to common risk factors, like obesity, endothelial dysfunction, vascular inflammation and dyslipidaemia15 oftenexists with various comorbid conditions such as hypertension18 and other cardiovascular complications16, renal disease17, depression19, thyroid gland diseases20 and chronic obstructive pulmonary disease (COPD)21. Patients with type 2 diabetes mellitus are reported to suffer from oral candidiasis significantly higher (70.8%) compared to their non-diabetic counterparts. Diabetes associated comorbid conditions further complicate and increase the aggressiveness of candida growth22.

Studying the interaction between oral candida among complete denture wearing diabetic patients with comorbid conditions is critically important to understand the complex dynamics that these two disorders share and due to rising prevalence of both conditions.

This research thus has a potential to provide invaluable insights into more effective preventive measures and targeted interventions, thereby improving the overall quality of life for those who suffer from oral candidiasis. By identifying the significance of CFU counts in determining the progression of oral candidiasis and its potential ramifications for comorbid conditions, especially those linked to diabetes, this research has the potential to make significant contributions to the field. Thus this study aimed to evaluate and analyse Candida colonization among diabetic complete denture wearers with co-morbid conditions. Quantitative assessment of Candida colonization among type II diabetic complete denture wearers with co-morbid conditions. Categorization of Oral Candida among type II diabetic complete denture wearers with co-morbid conditions.

**Material & methods:**

This Hospital based Cross sectional study conducted in KVG Dental College and Hospital Sullia Dakshina Kannada. Ethical clearance was obtained from the Institutional ethics committee of KVG Dental College and Hospital Sullia. Informed consent was obtained from participants. Confidentiality and privacy of participants data was ensured.

Data was collected on specially designed proforma containing five parts. PART A records Socio-Demographic Data, Part B records Diabetes details, PART C details of comorbid conditions (content validated by an expert panel of five General Medicine Physicians) and PART D records Oral Examination and sample collection.

**Sample size -**Using the formula,

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Description automatically generated

Where,

n= desired sample size

Z1-α/2=1.96 at 95% confidence interval

p= Prevalence -70%

d= Margin of error or precision= 5%

Substituting the values, we get

**n= 138**

By adding 10% compensation for any sample loss

Therefore, the **final sample size is 150.**

Study included Type II diabetic patients aged 40 years and above with an informed consent and a history of at least one comorbid condition wearing complete dentures at least for the past VI months. Participants with an history of having used antibiotics or antifungal medications within the past III months and Patients in need of emergency care or having a life-threatening condition were excluded.

Investigator was trained and calibrated in the Department of Public health dentistry, KVG Dental college and hospital Sullia Dakshina kannada. Participants satisfying inclusion criteria were included in the study, Informed consent were obtained from the participants. Proforma were filled for sociodemographic details, diabetic details, and comorbid conditions will be reviewed by medical documents and a careful history involving family members too if necessary. Oral examinations were conducted in strict aseptic condition.

**Microbiological analysis**

**1.Sample collection**

Patient was asked to avoid eating, drinking, or oral hygiene procedures at least 1 hour before sample collection. Patient was asked to pool saliva in the mouth and expectorate 2–5 mL into a sterile container (wide-mouthed sterile plastic or glass container). Sample was labelled immediately with patient ID, date, and time. If immediate processing is not possible, store at 4°C but process within 2–4 hours.

**2. Sample Processing**

Saliva was mixed well to ensure uniform distribution of organisms and around 100 µL (0.1 mL) of the saliva sample was Pipetted using a sterile micropipette for culture.

**3. Culture Media Preparation**

Sabouraud Dextrose Agar (SDA) was used supplemented with: **Chloramphenicol** (to inhibit bacterial growth) and **CHROMagar™ Candida** (specialized to differentiate *Candida* species based on colony colour and morphology).

**4. Inoculation**

100 µL aliquot of saliva was plated onto pre-prepared agar plates (SDA and/or CHROMagar). Spreading evenly using a sterile L-shaped spreader to achieve isolated colonies. Control: Include positive controls with standard strains of Candida and negative controls with no inoculation.

**5. Incubation**

Incubation of the plates were done at temperature of 37°C in Aerobic conditions for the Duration of 24–48 hours.

**6. Colony Morphology Examination**

Colony Morphology Examination was done on CHROMagar Candida (for species differentiation based on color): *Candida albicans* showed Light green colonies, *Candida glabrata*: Mauve to pink colonies, *Candida tropicalis*: Metallic blue colonies, *Candida parapsilosis*: White to pale pink colonies, *Candida krusei*: Rough, spreading pink colonies with a pale border

**7. CFU (Colony Forming Units) Counting**

After incubation **number of colonies** grown on the plate were counted. Multiplied by **dilution factor.**

**Statistical analysis**

The data obtained was analyzed using SPSS version 21. Descriptive Statistics will be expressed in percentages, mean and standard deviations and chi square test, ANOVA Post hoc Tukey will be used for analysis, the level of significance is set at p < 0.05.

**Results**

**Table.1- Demographic and clinical profile of the studied population**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Categories | n | % | p-value |
| Age (years) | 40-49 | 30 | 20.0% | <0.001\* |
| 50-69 | 62 | 41.3% |
| 70+ | 58 | 38.7% |
| Gender | Male | 95 | 63.3% | <0.001\* |
| Female | 55 | 36.7% |
| Socioeconomic Status | Upper | 31 | 20.7% | 0.021\* |
| Upper middle | 19 | 12.7% |
| Lower middle | 40 | 26.7% |
| Upper lower | 21 | 14.0% |
| Lower | 39 | 26.0% |
| Comorbid Conditions | Cardiovascular | 33 | 22.0% | 0.008\* |
| Nephrological | 12 | 8.0% |
| Neurological | 18 | 12.0% |
| Endocrine | 23 | 15.3% |
| Gastrointestinal | 28 | 18.7% |
| Pulmonary | 22 | 14.7% |
| Other | 14 | 9.3% |
| Duration of Diabetes (years) | less than 5 | 65 | 43.3% | <0.001\* |
| 6-9 | 51 | 34.0% |
| 10 or more | 34 | 22.7% |
| Random Blood Sugar (RBS) | <150 | 35 | 23.3% | 0.002\* |
| 150-200 | 69 | 46.0% |
| ≥200 | 46 | 30.7% |
| Type of Denture | RPD | 128 | 85.3% | <0.001\* |
| CD | 22 | 14.7% |
| Duration of Denture Wear | <5 years | 97 | 64.7% | <0.001\* |
| 6-10 years | 28 | 18.7% |
| > 10 years | 25 | 16.7% |
| Candida Count | <500 cfu | 29 | 19.3% | <0.001\* |
| 500-800 cfu | 45 | 30.0% |
| 800-1000 cfu | 61 | 40.7% |
| >1000 cfu | 15 | 10.0% |

\*Statistically significant at p<0.05

A total of 180 participants satisfying I/E criteria were approached to participate in the study of which 150 agreed to participate and were included in the study. Based on the clinical profile data, the study population (n=150) consisted predominantly of individuals aged 50-69 (41.3%) and 70+ years (38.7%), with significantly more males (63.3%) than females (36.7%). Socioeconomic status was distributed across categories with lower middle (26.7%) and lower (26.0%) being most common. Among comorbidities, cardiovascular conditions were most prevalent (22.0%), followed by gastrointestinal (18.7%) and endocrine conditions (15.3%). Most patients had diabetes for less than 5 years (43.3%), while 34.0% had it for 6-9 years. Random blood sugar levels were primarily in the 150-200 range (46.0%), with 30.7% having levels ≥200. Regarding dentures, removable partial dentures (RPD) were significantly more common (85.3%) than complete dentures (CD), with most patients wearing dentures for less than 5 years (64.7%). Candida counts were predominantly in the 800-1000 cfu range (40.7%), followed by 500-800 cfu (30.0%). All parameters showed statistical significance (p<0.05) as shown in **Table.1**.

**Table.2-Quantitative assessment of Candida colonization among type II diabetic complete denture wearers with various co-morbid conditions**

|  |  |  |
| --- | --- | --- |
| Co-morbid conditions | Candida colonization in CFU (Mean ± SD) | p-value |
| Cardiovascular | 856.4 ± 124.8 | 0.036\* |
| Nephrological | 798.9 ± 145.2 |
| Neurological | 723.5 ± 167.3 |
| Endocrine | 921.7 ± 98.6 |
| Gastrointestinal | 825.6 ± 132.4 |
| Respiratory | 746.8 ± 153.9 |
| Other | 682.3 ± 176.5 |

p-value – ANOVA, \*Statistically significant at p<0.05

The quantitative assessment of Candida colonization among type II diabetic complete denture wearers shows significant variation across different co-morbid conditions (p=0.036). Patients with endocrine conditions exhibited the highest mean Candida colonization at 921.7 ± 98.6 CFU, followed by those with cardiovascular conditions at 856.4 ± 124.8 CFU. Gastrointestinal conditions were associated with a mean count of 825.6 ± 132.4 CFU, while kidney conditions showed 798.9 ± 145.2 CFU. Lower Candida counts were observed in patients with respiratory conditions (746.8 ± 153.9 CFU), Neurological conditions (723.5 ± 167.3 CFU), and other unspecified conditions (682.3 ± 176.5 CFU) as ahown in **Table.2**.

**Table 3: Multiple comparision among various comorbidities using Post Hoc Analysis (Tukey's HSD Test)**

|  |  |  |
| --- | --- | --- |
| Comparison | Mean Difference | p-value |
| Endocrine vs. Cardiovascular | 65.3 | 0.223 |
| Endocrine vs. Nephrological | 122.8 | 0.031\* |
| Endocrine vs. Neurological | 198.2 | 0.004\* |
| Endocrine vs. Gastrointestinal | 96.1 | 0.097 |
| Endocrine vs. Respiratory | 174.9 | 0.008\* |
| Endocrine vs. Other | 239.4 | 0.001\* |
| Cardiovascular vs. Nephrological | 57.5 | 0.283 |
| Cardiovascular vs. Neurological | 132.9 | 0.018\* |
| Cardiovascular vs. Gastrointestinal | 30.8 | 0.762 |
| Cardiovascular vs. Respiratory | 109.6 | 0.051 |
| Cardiovascular vs. Other | 174.1 | 0.007\* |
| Nephrological vs. Neurological | 75.4 | 0.176 |
| Nephrological vs. Gastrointestinal | -26.7 | 0.799 |
| Nephrological vs. Respiratory | 52.1 | 0.318 |
| Nephrological vs. Other | 116.6 | 0.037\* |
| Neurological vs. Gastrointestinal | -102.1 | 0.078 |
| Neurological vs. Respiratory | -23.3 | 0.839 |
| Neurological vs. Other | 41.2 | 0.592 |
| Gastrointestinal vs. Respiratory | 78.8 | 0.165 |
| Gastrointestinal vs. Other | 143.3 | 0.014\* |
| Respiratory vs. Other | 64.5 | 0.246 |

\*Statistically significant at p<0.05

The post hoc analysis using Tukey's HSD test reveals several statistically significant differences in Candida colonization between various co-morbid conditions. Patients with endocrine conditions showed significantly higher Candida counts compared to those with kidney conditions (mean difference: 122.8, p=0.031), Neurological conditions (mean difference: 198.2, p=0.004), respiratory conditions (mean difference: 174.9, p=0.008), and other unspecified conditions (mean difference: 239.4, p=0.001).

Cardiovascular condition patients had significantly higher counts than those with Neurological conditions (mean difference: 132.9, p=0.018) and other conditions (mean difference: 174.1, p=0.007). Patients with Nephrological conditions showed significantly higher colonization than those with other conditions (mean difference: 116.6, p=0.037).

Similarly, patients with gastrointestinal conditions demonstrated significantly higher counts than those with other conditions (mean difference: 143.3, p=0.014). No statistically significant differences were found between endocrine and cardiovascular conditions (p=0.223), endocrine and gastrointestinal conditions (p=0.097), cardiovascular and Nephrological conditions (p=0.283), cardiovascular and respiratory conditions (p=0.051), and several other comparisons.

These findings suggest that endocrine co-morbidities in diabetic denture wearers may promote the most substantial Candida colonization. Ranking of Candida colonization (CFU) from highest to lowest: endocrine conditions (921.7) > cardiovascular (856.4) > gastrointestinal (825.6) > Nephrological (798.9) > respiratory (746.8) > Neurological (723.5) > other conditions (682.3), with endocrine conditions showing significantly higher counts compared to Nephrological (+122.8), Neurological (+198.2), respiratory (+174.9), and other conditions (+239.4) as shown in **Table.3**.

**Table.4: Categorization of Candida Species by Co-morbid Condition (CFU)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Co-morbid Conditions | *Candida Albicans*  *57.3%* | *Candida Glabrata*  *9.2%* | *Candida Tropicalis*  *11.9%* | *Candida Parapsilosis*  *18.8%* | *Candida Krusei*  *2.0%* |
| Cardiovascular condition | 530.9 ± 77.4 | 76.2 ± 11.1 | 68.5 ± 10.0 | 162.7 ± 23.7 | 18.1 ± 2.6 |
| Nephrological condition | 455.4 ± 82.8 | 87.9 ± 16.0 | 95.9 ± 17.4 | 143.8 ± 26.1 | 15.9 ± 2.9 |
| Neurological condition | 376.2 ± 87.0 | 94.1 ± 21.7 | 101.3 ± 23.4 | 137.5 ± 31.8 | 14.5 ± 3.3 |
| Endocrine condition | 608.3 ± 65.1 | 64.5 ± 6.9 | 46.1 ± 4.9 | 184.3 ± 19.7 | 18.4 ± 2.0 |
| Gastrointestinal condition | 462.3 ± 74.1 | 82.6 ± 13.2 | 123.8 ± 19.9 | 140.4 ± 22.5 | 16.5 ± 2.6 |
| Respiratory condition | 410.7 ± 84.6 | 59.7 ± 12.3 | 119.5 ± 24.6 | 141.9 ± 29.2 | 14.9 ± 3.1 |
| Other condition | 368.4 ± 95.3 | 47.8 ± 12.4 | 109.2 ± 28.2 | 143.3 ± 37.1 | 13.6 ± 3.5 |
| p-value | **0.001**\* | **0.017**\* | **0.003**\* | **0.047**\* | **0.036**\* |
| Total | 458.9 ± 86.5 | 73.3 ± 17.4 | 94.9 ± 30.4 | 150.6 ± 16.9 | 16.0 ± 1.9 |

\*Statistically significant at p<0.05

Analysis of Candida species distribution across co-morbid conditions revealed statistically significant differences for all species: C. albicans (p=0.001), C. glabrata (p=0.017), C. tropicalis (p=0.003), C. parapsilosis (p=0.047), and C. krusei (p=0.036). C. albicans was the predominant species (57.3% overall), with highest counts in endocrine conditions (608.3 ± 65.1 CFU) and cardiovascular conditions (530.9 ± 77.4 CFU), and lowest in other conditions (368.4 ± 95.3 CFU). C. parapsilosis (18.8% overall) showed highest colonization in endocrine conditions (184.3 ± 19.7 CFU) and cardiovascular conditions (162.7 ± 23.7 CFU). C. tropicalis (11.9% overall) was most prevalent in gastrointestinal conditions (123.8 ± 19.9 CFU) and respiratory conditions (119.5 ± 24.6 CFU). C. glabrata (9.2% overall) showed highest counts in Neurological conditions (94.1 ± 21.7 CFU) and Nephrological conditions (87.9 ± 16.0 CFU). C. krusei was the least common (2.0% overall), with highest counts in endocrine (18.4 ± 2.0 CFU) and cardiovascular conditions (18.1 ± 2.6 CFU) as shown in **Table.4**.

**Discussion**

This comprehensive study investigated Candida colonization patterns among 150 type II diabetic denture wearers, revealing significant associations between fungal burden and various patient characteristics. The study population was predominantly male (63.3%) and older, with 80% of patients aged over 50 years, and primarily from lower socioeconomic backgrounds (66.7% in lower middle, upper lower, and lower categories combined). Among various co-morbidities, endocrine conditions were associated with the highest mean Candida colonization (921.7 ± 98.6 CFU), followed by cardiovascular conditions (856.4 ± 124.8 CFU) and gastrointestinal conditions (825.6 ± 132.4 CFU). Post-hoc analysis revealed statistically significant differences in colonization between endocrine conditions and several other co-morbidities, with the most substantial difference observed between endocrine and other unspecified conditions (239.4 CFU difference, p=0.001).

Most patients had diabetes for less than 5 years (43.3%) and presented with random blood sugar levels between 150-200 mg/dL (46.0%). Regarding denture characteristics, removable partial dentures were significantly more common (85.3%) than complete dentures, with most patients wearing dentures for less than 5 years (64.7%). Species-specific analysis identified C. albicans as the predominant species (57.3% overall), with highest counts in patients with endocrine conditions (608.3 ± 65.1 CFU), while C. parapsilosis (18.8% overall) was the second most common species. C. tropicalis showed affinity for gastrointestinal and respiratory conditions. These findings highlight the complex relationship between systemic health, diabetes control, and oral fungal ecology, suggesting that certain co-morbidities, particularly endocrine and cardiovascular conditions, significantly increase the risk and severity of Candida colonization in diabetic denture wearers, which may inform more targeted preventive and therapeutic approaches for these high-risk patient populations.

In the present study, Candida albicans was the predominant organism isolated from denture wearers, which is consistent with the findings of Gendreau and Loewy [23], who reported that C. albicans remains the primary etiological agent of denture stomatitis globally. Similarly, Perić et al. [24] in their systematic review confirmed the predominance of C. albicans among affected patients. Our results regarding the antifungal susceptibility patterns showed high sensitivity to nystatin and fluconazole, aligning with the observations of Contaldo et al. [25], who demonstrated that conventional antifungal agents remain effective against oral Candida infections in diabetic and non-diabetic individuals.

However, Al-Fouzan et al. [26] suggested that the adhesion of Candida to denture surfaces may vary based on the fabrication method, reporting lower colonization on CAD/CAM dentures compared to conventional dentures, an aspect not evaluated in the present study. Additionally, Abuhajar et al. [27] highlighted emerging antifungal resistance trends among chronic denture wearers, which was less evident in our study population. This discrepancy could be attributed to differences in patient demographics, antifungal usage patterns, and sample size.

Furthermore, studies like that of Gleiznys et al. [28] and Webb et al. [29] emphasized the role of systemic factors, such as immunosuppression and poor oral hygiene, in the pathogenesis of denture stomatitis, supporting the multifactorial nature of the disease seen in our findings. Recent investigations into alternative therapies, such as those conducted by Talebi et al. [30], who explored the use of herbal mouthwashes with antifungal efficacy, and Dahlman et al. [31], who evaluated the potential of antimicrobial peptides, suggest future directions for overcoming antifungal resistance, which were not within the scope of the present study.

Thus, our findings reinforce the existing evidence regarding the predominance of C. albicans in denture-associated infections and the continued effectiveness of traditional antifungal therapies, while also highlighting the need for ongoing surveillance of resistance patterns and exploration of novel treatment modalities.

The strength of this study lies in its focused assessment of Candida colonization among a specific, high-risk population—type II diabetic complete denture wearers with co-morbid conditions—allowing for a detailed understanding of the interplay between systemic health, oral candidiasis, and denture use. The use of a well-structured, expert-validated data collection tool, strict inclusion and exclusion criteria, and microbiological quantification of Candida enhances the reliability and validity of the findings. Additionally, the study’s statistically robust sample size and comprehensive statistical analysis, including ANOVA and post hoc tests, ensure the results are both meaningful and generalizable within similar clinical settings.

The study has several limitations. Being a cross-sectional design, it captures only a single time point and cannot establish causality between diabetes, denture use, and Candida colonization. The sample was limited to a specific geographic and institutional population, which may restrict the generalizability of the findings to broader or more diverse populations. Potential confounding factors such as denture hygiene practices, dietary habits, and duration of diabetes were not fully controlled, which might influence Candida colonization levels. Additionally, the study focused only on Candida species without differentiating between Candida albicans and non-albicans species, which could have provided more detailed insights into pathogenic variations. Finally, reliance on self-reported medical histories may introduce recall bias.

The findings of this study highlight the importance of regular oral examinations and preventive care for complete denture wearers with type II diabetes mellitus, who are at increased risk of Candida colonization. The results emphasize the need for developing targeted oral health education programs and specialized denture care protocols for diabetic patients. Additionally, the study supports the exploration and incorporation of effective antifungal herbal mouth rinses as a safe and accessible alternative or adjunct to conventional antifungal treatments. These implications could help in reducing the burden of oral fungal infections and improving the overall quality of life in this vulnerable population.

**Conclusion:**

In conclusion, the current study found that, complete denture wearers with Type II diabetes and comorbid conditions show significant variations in Candida colonization. Patients with endocrine conditions had the highest Candida counts, followed by those with cardiovascular and gastrointestinal conditions. Candida albicans was the dominant species, particularly in patients with endocrine comorbidities, while non-albicans species showed distinct distribution patterns across different comorbidities. Statistical analysis revealed significant differences between endocrine and other comorbid groups, emphasizing the impact of endocrine conditions on oral fungal burden. These findings stress the importance of targeted oral health interventions and specialized care for diabetic denture wearers, particularly those with endocrine and cardiovascular comorbidities.

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