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COMPARATIVE ANALYSIS OF TWO ORAL BACTERIA (*PREVOTELLA INTERMEDIA* AND *TANNERELLA FORSYTHIA*) IN HYPERTENSIVE AND HEALTHY INDIVIDUALS IN MTHATHA, SOUTH AFRICA

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Abstract – *Objective:* Dysbiosis of the oral microbiota is considered a trigger for immuno-inflammatory processes at the root of many dysfunctions in the body. It is suspected in the etiology of arterial hypertension. However, few studies have investigated the potential impact of the oral microbiome on the etiology of hypertension. This work sought to evaluate the influence of *Prevotella intermedia* and *Tannerella forsythia* oral dysbiosis on the risk of hypertension in the South African population.

Patients and Methods: A case-control study was conducted involving saliva samples from 24 healthy participants and 23 hypertensive patients who attended the Gateway Clinic in Mthatha, Eastern Cape Province. DNA was extracted from the samples, and real-time PCR was conducted to detect the presence of target bacteria in both groups. The fold change in bacterial presence was then calculated for comparison.

Results: Findings showed that hypertensive participants consumed more coffee (38.30%) and were less physically active (46.81%) than non-hypertensives while suffering more dental problems. Although both target bacteria were present in both groups, only *P. intermedia* exhibited a notably greater abundance in the hypertensive group.

Conclusions: This study suggests that lifestyle habits, such as coffee consumption, lack of exercise, poor oral hygiene, and oral dysbiosis characterized by *P. intermedia*, could play a role in the onset or aggravation of hypertension, with potential implications for dental health.

Keywords: Hypertension, *Prevotella intermedia*, *Tannerella forsythia*, Oral microbiota, Comparative analysis.

INTRODUCTION

The oral cavity, like the urogenital tract, skin, respiratory tract, and gastrointestinal system, is home to extremely diverse microbiota essential to the body's proper functioning[1]. These microbial ecosystems, made up of complex communities of microbes and their genetic material[2], vary according

to the specific features of each body zone (intestine, skin, lungs, mouth). These microorganisms evolve due to numerous factors, such as diet, hygiene, age, genetics, migration, and the environment[1,3]. The oral flora, for example, consists mainly of bacteria capable of attaching themselves firmly to teeth and gums, enabling them to resist daily cleaning[4]. Imbalances in this flora have been associated with various non-communicable diseases, such as diabetes[5,6], cancer[7], inflammatory bowel disease[8], obesity[9], asthma[10], preterm birth[11], and many others.

Hypertension, a major global health problem, is one of the main risk factors for heart disease, such as stroke and heart failure[12]. It affects many people in South Africa, and government efforts are not stopping its progression. It is also frequently associated with co-morbidities such as obesity, type 2 diabetes, and chronic kidney disease[13]. While progress has been made in understanding the genetic and environmental factors influencing blood pressure[14], the relationship between hypertension and microbiota remains underexplored in South Africa, particularly concerning the oral microbiome.

Despite a limited number of studies on the link between the oral microbiome and hypertension[15,16], no research has yet targeted African populations, notably the Mthatha region in South Africa. This region, where socio-environmental factors play an important role, could influence microbiota composition and, consequently, affect the risk of developing hypertension. In this context, bacteria such as *Prevotella intermedia* and *Tannerella forsythia*, already known to be involved in periodontal disease, have not yet been sufficiently studied concerning this pathology. Due to their role in chronic inflammation, these bacteria could cause systemic invasions that can disrupt blood pressure regulation[17].

Thus, this research aims to fill this gap by analyzing the oral microbiota composition, focusing on *P. intermedia* and *T. forsythia*, in the context of hypertension. By studying these bacteria in healthy hypertensive individuals in Mthatha, this study aims to shed light on the potential relationship between the oral microbiome and blood pressure regulation while considering the specificities of the local population. This work sought to contribute to a better understanding of the microbial factors influencing hypertension, particularly in a uniquely African context.

Study Design

The study was conducted at Gateway Clinic and Walter Sisulu University's (WSU) Human Physiology Laboratory on the Nelson Mandela Drive (NMD) campus in Mthatha, Eastern Cape Province. This case-control study used convenience sampling to select 23 hypertensive patients (cases) and 24 non-hypertensive participants (controls) at the Gateway Clinic.

Inclusion Criteria

This study included participants aged 18 and older, both male and female, with a confirmed diagnosis of hypertension, as well as non-hypertensive individuals. Before their inclusion, the blood pressure of control participants was measured to confirm it was within the normal range, ensuring their non-hypertensive status was not mistakenly attributed to a lack of medical consultation.

Exclusion Criteria

The study excluded nursing and pregnant women, people currently or who had used antibiotics in the past three months, as well as diabetic and asthmatic patients. Additionally, individuals with chronic conditions, who had not signed a consent form, who had received periodontal treatment in the past six months, who were suffering from severe immunosuppression, or who had an acute infection at the time of enrolment were also excluded.

Anthropometric Measurements

Height was measured using a TCS-200-RT stadiometer. The participant was instructed to remove shoes and bulky clothing, ensuring their head, heels, buttocks, and shoulder blades were in

contact with the stadiometer. The feet were positioned with a small gap between the legs, the feet pointing straight ahead, and the ear canal aligned with the cheekbone. The horizontal arm of the stadiometer was adjusted to rest gently on top of the participant's head, and height was recorded to the nearest centimeters. Weight, body fat percentage (%), and muscle percentage (%) were measured using an Omron BF511 digital scale. Participants were instructed to remove heavy outer clothing and shoes and empty their pockets. They were instructed to stand still in the center of the platform with a 10 cm gap between their heels to ensure weight distribution across both legs. Weight was recorded in kilograms.

Saliva Sample Collection

Unstimulated saliva was collected from all participants following the protocol described by Jo et al[18]. Before collection, participants rinsed their mouths with mineral water to remove food debris, then waited approximately 30 minutes to allow natural saliva production and minimize sample dilution. A minimum of 1 mL of saliva was collected from participants by allowing it to pool in the mouth and spit into a sample tube. Samples were immediately stored in a cooler containing ice and transported to the laboratory, where they were stored at -80°C within 3 hours of collection to ensure DNA stability for subsequent analysis.

DNA Extraction and Quantification

According to the manufacturer's protocol, DNA extraction from saliva samples was performed using the ZymoBIOMICSTM DNA Extraction Miniprep Kit. Then, DNA concentration and purity were assessed using a Nanodrop spectrophotometer (Thermo Scientific, NND-1 ND-ONE-W-1PR22, Thailand). To prevent degradation by chemical and enzymatic processes, extracted DNA was stored as a precipitate in ethanol at -80°C[19].

Probe or Primer Sets for Real-Time PCR

The species-specific region on the 16S rRNA was used to design the TaqMan probe and primer sets (Table 1) and were utilized to detect *P. intermedia* and *T. forsythia*. Additionally, as reported by Barbadoro et al[20], the total amount of selected eubacterial species in the specimens was quantified using a universal primer pair that was based on the conserved region of the 165S rRNA gene. The primers and TaqMan probes were ordered and synthesized from Inqaba biotecTM (South Africa).

Amplification of DNA Through Real-Time PCR

Real-time PCR was performed using a CFX 96 Real-Time System, and all samples were run in duplicates. Each PCR was carried out in a total volume of 20 µL, consisting of 10 µL of Luna Uni-

TABLE 1. SEQUENCES OF PRIMERS AND PROBES USED REAL-TIME PCR[20].

Bacteria	Primers and probes
Prevotella intermedia	5′-TCCACCGATGAATCTTTGGTC-3′ 5--ATCCAACCTTCCCTCCACTC-3′ 5′-FAM-CGTCAGATGCCATATGTGGACAACATCG-TAMRA-3′
Tannerella forsythia	5′-AGCGATGGTAGCAATACCTGTC-3′ 5′-TTCGCCGGGTTATCCCTC-3′ 5′-FAM-TGAGTAACGCGTATGTAAACCTGCCCGC-TAMRA-3′
Universal	5′-TCCTACGGGAGGCAGCAGT-3′ 5′-GGACTACCAGGGTATCTAATCCTGTT-3′ 5′-FAM-CGTATTACCGCGGCTGCTGGCAC-TAMRA-3′

versal Probe qPCR Master Mix, 0.8 µL each of forward and reverse primers, 0.4 µl of probe, 2 µL of template DNA solution and an appropriate dose of sterilized Nuclease-free water. Amplification was conducted on the CFX 96 Real-Time System using a specified thermocycling program. At the annealing temperature, a fluorescence signal was observed to measure intensity. Fluorescence data was analyzed using CFX Manager Dx 3.1 (v3.1.3090.1022) Win C216125 software. The theoretical cell numbers were determined by calculating the bacterial DNA level through qRT-PCR, as previously described by Barbadoro et al[20].

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki. The Research and Ethics Committee of the Faculty of Medicine and Health Sciences, Walter Sisulu University granted ethical approval for the study (protocol code 012/2022; Approval Date: 23 February 2022) for ethical and biosafety clearance. Before taking part in the study, each participant signed an informed consent form after being fully briefed on our research's aims and challenges. To ensure confidentiality, unique codes were assigned to each participant, replacing the use of their names.

Statistical Analysis

Data were initially captured using Microsoft Excel, then cleaned and transferred to Stata 18 for data analysis. The mean and standard deviation of frequency distributions and descriptive statistics were examined. Ct values were determined for each bacterium. The ΔΔCt was then calculated to assess the relative change in gene expression. To do this, the relative delta-delta method was applied, allowing Cq values to be converted into relative quantities, which aligns with the method described by Pfaffl[21]. An unpaired *t*-test was applied to assess differences in bacterial expression and quantification between the two groups. Results were considered statistically significant if the *p*-value was 0.05 or less.

RESULTS

Demographic Characteristics of the Study Population

This study involved forty-seven participants from the Gateway Clinic in Mthatha, in the Eastern Cape province. The mean age of the participants was 44.57 ± 11.70 years, with an age range of 18 to 80 years. The participants were divided into two groups: hypertensive (n=23, 48.9%) and non-hypertensive (n=24, 51.06%). Approximately 68.04% of the participants were female, while 32.92% were male. Most participants (53.19%) were between 40 and 80 years old, while 46.81% were between 18 and 40 years old. Most participants were married (59.58%) and lived in rural areas (55.32%). A significant proportion of participants had completed high school or higher education (82.98%), while only 17.02% had attended primary school. About 57.44% of participants were employed. Regarding body weight, 72.34% of participants were overweight, while 27.66% had a normal weight (Table 2).

Lifestyle and Oral Health Habits Parameters

Table 3 reveals significant differences between hypertensive and non-hypertensive participants regarding their lifestyle and oral health habits. Hypertensive participants had a greater propensity to consume coffee (38.30%) and avoid physical exercise (46.81%) than non-hypertensive, with only 2.13% of hypertensives engaged in physical activity. What's more, a high proportion of hypertensive participants had dental problems, with 68.09% complaining of missing teeth and 59.57% of tooth decay. In contrast, non-hypertensives had a higher rate of dental consultations (31.91%).

TABLE 2. SOCIO-DEMOGRAPHIC CHARACTERISTICS DIFFERENCE AMONG ESSC AND HEALTHY CONTROLS.

Variables	Categories	Hypertensive n (%)	Non-hypertensive n (%)	Total (%)
Age (Years)	18-40	3 (6.38)	19 (40.43)	22 (46.81)
	40-80	20 (42.55)	5 (10.64)	25 (53.19)
Gender	Female	21 (44.64)	11 (23.40)	32 (68.04)
	Male	2 (4.26)	13 (27.66)	15 (32,92)
Marital status	Single	13 (27.66)	6 (12.77)	19 (40.43)
	Married	10 (21.28)	18 (38.30)	28 (59.58)
Place of residence	Village	16 (34.04)	10 (21.28)	26 (55.32)
	Township	7 (14.89)	14 (29.79)	21 (44.68)
Education level	Primary	7 (14.89.00)	1 (2.13)	8 (17.02)
	HS &Tertiary	16 (34.04)	23 (48.94)	39 (82.98)
Employment	Yes	7 (14.89)	20 (42.55)	27 (57.44)
	No	16 (34.04)	4 (8.51)	20 (42.55)
BMI (Kg/m^2)	Normal	1 (2.13)	12 (25.53)	13(27.66)
	Overweight	22 (46.81)	12 (25.53)	34(72.34)

HS: High School; BMI: body mass index.

TABLE 3. LIFESTYLE AND ORAL HEALTH HABITS IN HYPERTENSIVE *VS.* NON-HYPERTENSIVE PARTICIPANTS.

Variables of interest	Categories	Hypertensive n (%)	Non-hypertensive n (%)	Total (%)
Coffee consumption	Yes	18 (38.30)	10 (21.28)	28 (59.58)
	No	5 (10.64)	14 (29.79)	19 (40.43)
Physical exercise	Yes	1 (2.13)	8 (17.02)	9 (19,15)
	No	22 (46.81)	16 (34.04)	38 (80.85)
Alcohol consumption	Yes	4 (8.51)	11 (23.40)	15 (31.91)
	No	19 (40.43)	13 (27.66)	32 (68.09)
Smoking	Yes	2 (4.26)	4 (8.51)	6 (12.77)
	No	21 (44.68)	20 (42.55)	41 (87.23)
Teeth brushing per day	1/day	11 (23.40)	11 (23.40)	22 (46.80)
	>1/day	12 (25.53)	13 (27.66)	25 (53.19)
Oral health history	None	9 (19.15)	24 (51.06)	33 (70.21)
	Yes	14 (29.79)	0 (00.00)	14 (29.79)
Missing teeth	None	1 (2.13)	14 (29.79)	15(31.92)
	Once	22 (46.81)	10 (21.28)	32 (68.09)
Tooth decay	Once had	13 (27.66)	15 (31.91)	28 (59.57)
	Never	10 (21.28)	9 (19.15)	19 (40.43)
Dentist visit	Once	10 (21.28)	15 (31.91)	40 (53.19)
	Never	13 (27.66)	9 (19.15)	22 (46.81)

Presence and Abundance of Selected Bacteria

Figure 1 illustrates the presence and abundance of selected bacteria in participants. Analysis of the results shows that *T. forsythia* and *P. intermedia* were detected in all saliva samples from both hypertensive and non-hypertensive participants. In the non-hypertensive participants, the mean abundance of *P. intermedia* was 6.99±0.60, while in the hypertensive group, it was slightly higher, at 8.58±0.70 ($p = 0.04$). On the other hand, the abundance of *T. forsythia* was similar in both groups: it was 11.13±0.96 in the non-hypertensives and 11.27±1.25 in the hypertensives ($p = 0.97$), suggesting a stability of this bacteria in both populations.

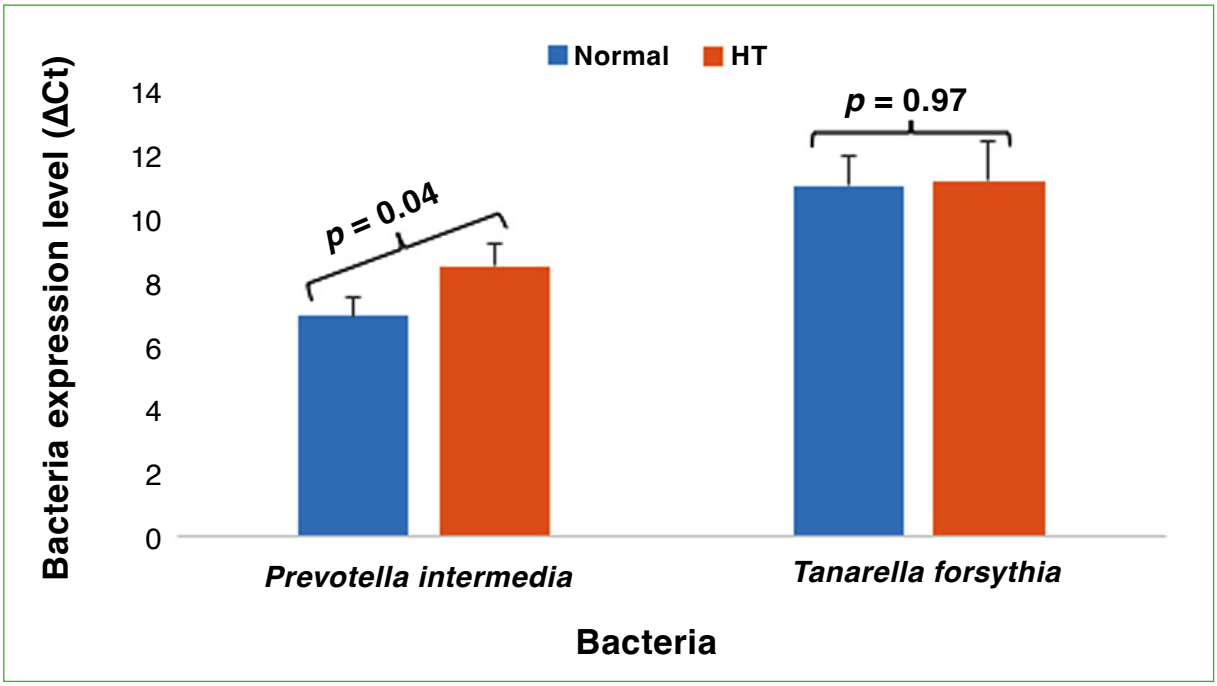

Figure 1. Bar graph shows *Tanarella forsythia* and *Prevotella intermedia* average relative abundance in normal and hypertensive participants. Normal distribution values are represented as mean±SD, $p \leq 0.05$ is considered statistical significance. HT: hypertensive; Normal: non-hypertensive.

Relative Fold Change

Table 4 shows the relative change in bacterial abundance for *P. intermedia* and *T. forsythia*. The results show that *P. intermedia* abundance decreased in non-hypertensive compared to hypertensive with a relative change of 0.364. In contrast, *T. forsythia* abundance remained almost unchanged, with a relative change of 0.988 in both groups.

DISCUSSION

The oral cavity is home to billions of microorganisms, and an imbalance in the oral bacterial flora, known as dysbiosis, can lead to significant changes in certain body tissues[22]. This study highlights significant differences between the hypertensive and non-hypertensive groups, particularly concerning lifestyle and oral health behaviors. Hypertensive participants tended to consume more coffee (38.30%) and avoid physical exercise (46.81%) than their non-hypertensive counterparts. This lack of physical activity is of particular concern, as it is widely recognized that exercise plays a fundamental role in managing hypertension and preventing various chronic pathologies[23,24]. The high rate of overweight (72.34%) observed in this study illustrates the direct impact of lack of exercise on physical health. Being overweight is a well-documented risk factor for hypertension and oral diseases[25,26].

Moreover, excessive coffee consumption represents an additional risk, particularly for dental health. Much more than just a factor in tooth discoloration, coffee exposes the oral cavity to high

TABLE 4. AVERAGE RELATIVE FOLD CHANGE OF BACTERIA BETWEEN HYPERTENSIVE AND NON-HYPERTENSIVE GROUPS.

Bacteria	Relative fold change
Prevotella intermedia	0.364
Tanarella forsythia	0.988

temperatures, as it is often consumed hot, and to active ingredients capable of causing bacterial mutations. These previously harmless bacteria then become pathogenic, increasing the risks to oral health and potentially leading to systemic effects with the consequence of disrupting the function of certain organs, including blood vessels[27]. It is also important to note that the caffeine contained in coffee has a detrimental effect on blood pressure. Recent studies, such as those by Surma and Oparil[15] and Islam et al[28], have shown that excessive coffee consumption is linked to higher blood pressure.

Similarly, the high number of dental oral disorders in hypertensive participants compared to non-hypertensive patients further underlines the interconnection between health behaviors, lifestyle, and chronic disease. Variations in the presence and quantity of specific bacteria could provide an explanation for this. The results showed that both hypertensive and non-hypertensive participants were present in all saliva samples in *T. forsythia* and *P. intermedia*. This presence in both groups suggests that these bacteria are part of the natural habitat of the oral cavity. These findings are corroborated by the work of Könönen et al[16], who observed that these bacteria are present in low concentrations in the oral cavity of infants, but their abundance increases in healthy adults and even more so under pathological conditions, such as periodontal diseases.

However, notable differences are observed in the abundance of *P. intermedia* between the two groups with a statistically significant difference ($p = 0.04$). This result is in line with the work of Goodrich et al[29], who reported a higher abundance of this bacterium in hypertensive subjects compared with non-hypertensive subjects. This increased abundance of *P. intermedia* in hypertensive patients suggests that this bacterium may be associated with hypertension, although other factors need to be explored to better understand this relationship. According to Priyamvara et al[30], bacteremia could be the key mechanism linking dysbiosis of the oral microbiome to cardiovascular disease. This is because oral pathogens, such as *T. forsythia*, *P. intermedia*, and *P. gingivalis*, have been detected in atheromatous plaques, suggesting bacterial translocation from the mouth to the system[31,32]. These bacteria release lipopolysaccharides (LPS), endotoxins that trigger inflammation[33], contributing to atherosclerosis and increasing the risk of stroke[34].

In addition, poor oral hygiene, common in hypertensive patients, promotes the accumulation of oral bacteria and the onset of periodontal disease, which has a known effect on hypertension[35,36]. Similarly, antihypertensives (beta-blockers and diuretics), can reduce salivary flow and alter oral pH, thus disrupting the oral microbiome[37]. Analysis of relative variation in abundance confirms these observations. The abundance of *P. intermedia* decreased in non-hypertensive compared to hypertensive participants, with a relative change of 0.364, confirming that hypertension could be linked to an increase in the presence of this bacteria.

In contrast, the abundance of *T. forsythia* showed no significant differences between the two groups. This stability of *T. forsythia* abundance in both groups suggests that the hypertensive status of the participants in the sample studied does not influence this bacterium. However, factors such as diet, genetics, smoking, and oral hygiene affect *T. forsythia* levels, complicating its exact role in hypertension[38].

CONCLUSIONS

In conclusion, this study reveals that excessive coffee consumption, lack of exercise, and more frequent dental problems are lifestyle behaviors closely linked to hypertension in the Eastern Cape of South Africa. Compared with healthy controls, the differential abundance of *P. intermedia* in hypertensive individuals suggests a potential relationship between oral microflora and hypertension. However, no change in *T. forsythia* levels in either group was observed in the study population. These findings underline the importance of good oral hygiene and a healthy lifestyle in preventing the risks associated with hypertension. Future longitudinal studies with repeated clinical visits are needed to examine changes in the oral microbiome over time.

Limitations and Strengths of the Study

This research has several limitations. It did not consider the pH of supragingival biofilm, which can influence bacterial growth. Additionally, most participants had good oral hygiene, which may not

represent the general population. The qPCR method used in this study cannot distinguish between live and dead cells, necessitating confirmation of results with culture-based methods, such as the plate count method. This study, despite its limitations, offers valuable insights into the relative abundance of oral bacteria in hypertensive individuals. The findings highlight the potential link between oral microbiota and hypertension, underscoring the importance of further research to better understand the mechanisms underlying this association.

Author Contributions

This work was made possible thanks to the collaboration of all the authors. The final version of the manuscript, as published, has been read and approved by all the authors.

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Informed Consent Statement

All subjects involved in the study gave their informed consent.

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Conflicts of Interest

The authors have declared no conflicts of interest.

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Data availability

No datasets were generated or analyzed during the current study.

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