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THE RELATIONSHIP BETWEEN ORAL HEALTH AND HORMONAL STATUS IN PRESCHOOL CHILDREN WITH AUTISM SPECTRUM DISORDER

DOCTORAL DISSERTATION

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POVEZANOST IZMEĐU ORALNOG ZDRAVLJA I HORMONSKOG STATUSA U DJECE PREDŠKOLSKE DOBI S POREMEĆAJEM IZ AUTISTIČNOG SPEKTRA

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I see this work as a document of my personal growth and a contribution to the wider community, demonstrating that resilience and continuous learning can transform personal challenges into opportunities for progress.

Summary

THE RELATIONSHIP BETWEEN ORAL HEALTH AND HORMONAL STATUS IN PRESCHOOL CHILDREN WITH AUTISM SPECTRUM DISORDER

The scientific literature on the relationship between oral health and hormonal status in preschool children with autism spectrum disorder (ASD) is limited. This doctoral dissertation aimed to evaluate salivary cortisol and melatonin levels in relation to oral health parameters and the presence of *Candida albicans*. The sample comprised 90 children, 45 with ASD and 45 healthy controls aged 4-6 years. Data were collected using an adapted WHO questionnaire, alongside clinical oral examinations assessing the dmft index, GI, PI, and BEWE. Buccal mucosa samples were collected to identify *C. albicans*. Saliva samples were obtained and analysed for cortisol and melatonin levels by means of an enzyme-linked immunosorbent assay. No significant differences in oral health status were observed between the two groups. Oral health-related quality of life indicated significant differences within the ASD group, with higher Parental–Caregiver Perceptions Questionnaire 16-item Short Form scores affecting 'emotional well-being', 'functional limitations', and 'social well-being' ($p < 0.001$), while 'oral symptoms' displayed no significant difference. Furthermore, children with ASD had fewer dental visits, primarily for toothache, brushed their teeth less frequently ($p = 0.035$), and experienced increased behavioural difficulties during dental appointments ($p < 0.001$). *C. albicans* was detected in 35.6% of children with ASD compared to 11.1% of the controls, with significantly higher colony counts in the ASD group. Moreover, children with ASD exhibited elevated salivary cortisol levels ($p < 0.001$) and reduced melatonin concentrations ($p = 0.04$). Nevertheless, no significant correlations were found between *Candida* load and hormone levels. Enhancing oral health through the education of parents and caregivers could improve the OHRQoL and overall well-being of children with ASD. These children showed increased colonisation by *C. albicans*, elevated cortisol levels, and reduced melatonin levels compared to controls. While no direct links between hormone levels and fungi were found, these results suggest a possible imbalance in the nervous system, hormones, and microbes in ASD. These findings contribute to a better understanding how oral health and hormone changes interact in children with ASD. Future studies with larger groups and long-term designs will help confirm these findings and create personalized oral hygiene therapy.

Keywords: autism spectrum disorder; salivary cortisol; salivary melatonin; *Candida albicans*; oral health-related quality of life; plaque index; gingival index

Sažetak

POVEZANOST IZMEĐU ORALNOG ZDRAVLJA I HORMONSKOG STATUSA U DJECE PREDŠKOLSKE DOBI S POREMEĆAJEM IZ AUTISTIČNOG SPEKTRA

Cilj: Poremećaj iz autističnog spektra, ASD, neurološki je razvojni poremećaj koji može utjecati na oralno zdravlje i kvalitetu života. Djeca s ASD-om često se suočavaju s poteškoćama u održavanju oralne higijene jer senzorna osjetljivost može učiniti četkanje zubi neugodnim, što dovodi do izbjegavanja ili neredovitog četkanja zubi i povećava rizik od karijesa. Osim toga, djeca s ASD-om mogu pokazivati promijenjen odgovor na stres i cirkadijalnu regulaciju, što se odražava kroz razinu kortizola i melatonina u slini, kao i moguće promjene u oralnoj mikrobnoj ravnoteži, uključujući kolonizaciju *Candidom albicans*. Cilj ove studije bio je procijeniti razinu kortizola i melatonina u slini u odnosu na parametre oralnog zdravlja i prisutnost *Candide albicans* kod djece s ASD-om u dobi od 4 do 6 godina u usporedbi sa zdravom djecom.

Ispitanici i metode: U Prištini je provedena presječna komparativna studija koja je obuhvatila 90 djece u dobi od 4 do 6 godina, od čega 45 djece s dokumentiranom dijagnozom poremećaja iz autističnog spektra bez intelektualnog teškoća i 45 neurotipičnih kontrolnih ispitanika istog spola. Djeca s ASD-om regrutirana su iz specijaliziranih rehabilitacijskih i psiholoških službi, dok su kontrolni ispitanici upisivani iz javnih predškolskih ustanova, a u obje skupine primijenjen je isti protokol i alati za procjenu. Status oralnog zdravlja procijenio je jedan kalibrirani ispitivač prema metodologiji Istraživanja oralnog zdravlja Svjetske zdravstvene organizacije, koristeći indeks karijesnih, nedostajućih i plombiranih mliječnih zuba, indeks gingive, indeks plaka i osnovni pregled erozivnog trošenja. Kako bi se poboljšala suradnja kod djece s ASD-om, klinički postupak je prilagođen ponašanju. Roditelji ili skrbnici ispunili su prilagođeni upitnik WHO-a o oralnom zdravlju i P-CPQ-16. Brisevi bukalne sluznice prikupljeni su za detekciju i kvantifikaciju *Candide albicans* kulturom na Sabouraud dekstroznom agaru, s potvrdom vrste na kromogenom ili biokemijskom mediju. Nestimulirana večernja slina prikupljena je pomoću Salivette® uređaja i analizirana na koncentracije kortizola i melatonina enzimsko-imunosorbentnim testom. Podaci su analizirani korištenjem deskriptivne statistike, hi-kvadrat ili Fisherovog egzaktnog testa, Mann-Whitneyjevog U testa, Spearmanove korelacije, multiple regresije i binarne logističke regresije, pri čemu se vrijednost $p < 0,05$ smatrala statistički značajnom. Etičko odobrenje dobiveno je od relevantnih institucija, a pisani informirani pristanak osiguran je od roditelja ili skrbnika.

Rezultati: Između dviju skupina nisu utvrđene značajne razlike u statusu oralnog zdravlja, uključujući dmft, GI, PI i BEWE. Međutim, kvaliteta života povezana s oralnim zdravljem pokazala je značajne razlike unutar skupine djece s ASD-om. Viši rezultati na kratkoj verziji Upitnika o percepciji roditelja i skrbnika od 16 čestica ukazivali su na izraženiji utjecaj u domenama emocionalne dobrobiti, funkcionalnih ograničenja i socijalne dobrobiti ($p < 0,001$), dok u domeni oralnih simptoma nije zabilježena značajna razlika. Nadalje, djeca s ASD-om imala su manje stomatoloških posjeta, najčešće zbog zubobolje, rjeđe su prala zube ($p = 0,035$) te su pokazivala izraženije poteškoće u ponašanju tijekom stomatoloških pregleda ($p < 0,001$). Nisu zabilježene razlike u uporabi zubne paste s fluorom, stanju gingive, iskustvu zubobolje ni prehrambenim navikama. Djeca s ASD-om pokazivala su veće poteškoće pri konzumaciji tvrde hrane ($p = 0,029$) i veću učestalost samoozljeđujućeg ponašanja ($p \leq 0,010$).

Candida albicans otkrivena je u 35,6 % djece s ASD-om u usporedbi s 11,1 % djece iz kontrolne skupine ($p = 0,006$), uz značajno veći broj kolonija u skupini djece s ASD-om. Osim toga, djeca s ASD-om imala su povišene razine salivarnog kortizola ($p < 0,001$) i snižene koncentracije melatonina ($p = 0,04$). Ipak, nisu utvrđene značajne korelacije između opterećenja kandidom i razina hormona. Dob je pokazala blagu tendenciju kao prediktor razine kortizola, ali bez statističke značajnosti. Linearne regresijske analize pokazale su da prisutnost kandide predviđa blago, ali statistički neznačajno povećanje dmft indeksa i razine kortizola ($p \approx 0,057$ odnosno $p \approx 0,112$), čime se dodatno potvrđuje izostanak snažnih povezanosti između navedenih varijabli.

Zaključak: Zaključak: Iako nisu pronađene značajne razlike u kliničkom oralnom statusu između djece s poremećajem iz autističnog spektra i kontrolne skupine, djeca s ASD-om pokazala su značajno lošiju kvalitetu života povezanu s oralnim zdravljem, posebno u funkcionalnoj, emocionalnoj i socijalnoj domeni. Također su rjeđe posjećivali stomatologa, češće zbog boli, obavljali su manje redovitu oralnu higijenu i pokazivali izraženije poteškoće u ponašanju tijekom stomatoloških pregleda. Skupina djece s ASD-om također je imala značajno veću oralnu kolonizaciju vrstom *Candida albicans*, povećanu razinu kortizola u slini i smanjenu razinu melatonina u usporedbi s kontrolnom skupinom. Iako hormonski parametri nisu bili neovisni prediktori gljivične kolonizacije, njihova istovremena prisutnost može ukazivati na specifično oralno i neuroendokrino okruženje koje bi moglo doprinijeti narušenoj oralnoj ravnoteži kod djece s ASD-om. Rezultati ističu potrebu za ciljanim preventivnim programima koji uključuju edukaciju roditelja i skrbnika, strukturiranu podršku u održavanju oralne higijene, kontrolu plaka i gljivične kolonizacije te stomatološko okruženje prilagođeno senzornim i bihevioralnim potrebama djece s ASD-om. Potrebne su buduće longitudinalne i

intervencijske studije s većim uzorcima kako bi se potvrdili ovi nalazi, utvrdili uzročno-posljedični odnosi i razvile personalizirane strategije oralne njege za ovu ranjivu populaciju.

Ključne riječi: poremećaj iz spektra autizma; salivarni kortizol; salivarni melatonin; *Candida albicans*; kvaliteta života povezana s oralnim zdravljem; indeks plaka; gingivni indeks.

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List of abbreviations

ASD	Autism Spectrum Disorder
BEWE	Basic Erosive Wear Examination
C. albicans	Candida albicans
CFU	Colony-Forming Units
dmft	decayed, missing, and filled teeth index
ECC	Early Childhood Caries
ELISA	Enzyme-Linked Immunosorbent Assay
GI	Gingival Index
HPA	Hypothalamic-Pituitary-Adrenal axis
IQR	Interquartile Range
M	Mean
N	Number of participants / sample size
OHI	Oral Hygiene Instructions or Index?
OHRQoL	Oral Health-Related Quality of Life
OR	Odds Ratio
P-CPQ-16	Parental-Caregivers Perceptions Questionnaire 16-item Short Form
PI	Plaque Index
p	Probability value
r	Correlation coefficient
SDA	Sabouraud Dextrose Agar
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

1. INTRODUCTION

1.1. Definition and Epidemiology of Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterised by persistent difficulties in social interaction and communication, alongside restricted interests and repetitive behaviours (1). The condition is marked by heterogeneity; some individuals live independently, while others require substantial support throughout their lives (2). Comorbid intellectual disability, sensory hypersensitivity or hyposensitivity, and sleep disturbances are frequently reported (3). Early recognition and support are strongly associated with superior long-term outcomes (2,4). Nevertheless, numerous individuals with autism remain undiagnosed or receive a diagnosis late, delaying access to interventions that could reduce the impact on learning and socio-emotional development (5-7).

Reliable epidemiological estimates are essential for planning health and social services. Global Burden of Disease estimates indicate that approximately 1% of the population is autistic (8). Surveillance in high-income countries suggests even higher prevalence rates among children, likely reflecting greater awareness and improved diagnostic practices (9,10). For instance, in the Autism and Developmental Disabilities Monitoring (ADDM) Network report using 2022 data, ASD prevalence among 8-year-olds across 16 sites was 32.2 per 1,000, presented as “one in 31 (11). These rising numbers do not signify a sudden epidemic but rather a result of broadened diagnostic criteria and enhanced detection across the spectrum. Despite this, access to services remains uneven, and several families lack the support they require (5).

Terminology has evolved alongside shifts in understanding. Although ‘autism spectrum disorder’ remains the formal diagnostic term, the preference for ‘autism spectrum condition’ is increasing in order to affirm neurodiversity and reduce stigma (12). This transition is significant as language influences attitudes towards individuals with autism and potentially affects the design and delivery of services.

1.2. Oral Health in Children with ASD

1.2.1. Clinical Oral Health Status

Oral health is integral to overall well-being and quality of life, oral diseases are among the most prevalent conditions worldwide and substantially affect quality of life and economic burden. For children and adolescents on the autism spectrum, maintaining good oral health can be particularly challenging owing to sensory sensitivities, communication differences, and behavioural patterns that disrupt routine hygiene practices (13,15). A growing body of research has examined the dental status of individuals with autism. Systematic reviews and meta-analyses indicate a generally higher prevalence of dental caries in children with autism than in their neurotypical peers, although the magnitude of this disparity varies across studies and populations (13-15). Certain investigations have reported similar caries rates, emphasising the importance of contextual factors such as diet, socio-economic status, and access to healthcare (16-18).

Dental caries results from acid production by oral biofilms when bacteria metabolise fermentable carbohydrates (16). In children with autism, selective eating behaviours often favour carbohydrate-rich foods and sugary snacks. Combined with irregular brushing and difficulties with flossing, this increases exposure to cariogenic substrates (19). Salivary factors may also play a significant role. Studies have indicated altered baseline pH and buffering capacity in some children with autism, potentially contributing to enamel demineralisation (18). Meta-analyses have demonstrated that individuals with autism tend to have higher plaque and gingival indices, suggesting poorer oral hygiene and an increased risk of periodontal disease. Gingival inflammation and bleeding are frequently observed (13,15).

In addition to caries and gingivitis, children with ASD may experience malocclusions, bruxism, enamel hypoplasia, and traumatic dental injuries (13,15,20). Sleep bruxism appears to be more prevalent among children with autism, and has been associated with sleep disturbances and anxiety (21,22). This can lead to wear facets, chipped teeth, and temporomandibular joint strain, further compromising oral function. The heterogeneity of findings underscores the necessity for context-specific data, as socio-economic status, dietary patterns, healthcare systems, and cultural practices can all modulate caries risk and periodontal health (13,15,17).

1.2.2. Pathophysiology of Dental Disease and Microbial Dysbiosis

Caries is driven by a shift in the composition and metabolic activity of dental biofilms. In early childhood, acidogenic bacteria, such as *Streptococcus mutans* (*S. mutans*), colonise the tooth surface and produce extracellular polysaccharides that facilitate biofilm formation (16,23). When sugar intake is frequent and oral hygiene is inadequate, these biofilms maintain a low pH that favours aciduric bacteria, and contributes to enamel demineralisation (16). Studies have revealed that children with autism often exhibit an increased abundance of *S. mutans* and reduced diversity of commensal species (18,24). Salivary cytokine profiles suggest that neuroimmune dysregulation may further influence microbial ecology (18).

An important aspect of dysbiosis in early childhood caries is the synergy between bacteria and fungi. *Candida albicans*, a component of the normal oral flora, can become pathogenic under specific conditions. Research has demonstrated that *C. albicans* can form cross-kingdom biofilms with *S. mutans*, which exhibit greater virulence than monomicrobial biofilms (25,26). The fungal mannans facilitate the binding of *S. mutans* glucosyltransferases, thereby enhancing the production of extracellular glucans and contributing to the acidification of the microenvironment (27). *In vitro* experiments indicate that *C. albicans* metabolites stimulate *S. mutans* microcolony formation and acid production (28), while saliva and dietary sugars modulate biofilm development (29). These cross-kingdom interactions have been implicated in severe early childhood caries, and may be particularly relevant in children with altered salivary flow or immune function (23,25,26). Chronic inflammation and immune dysregulation have been widely reported in autism spectrum disorder and may predispose individuals to microbial imbalance and fungal overgrowth (30). Fungal dysbiosis may extend beyond the oral cavity, and studies examining gut microbiota in children with autism have reported increased yeast colonisation (31). Meta-analyses also confirm an association between Candida albicans and the severity of early childhood caries (32). However, the causal relationships remain uncertain and are probably bidirectional, as dysbiosis may also contribute to gastrointestinal and behavioural symptoms.

1.2.3. Early Childhood Caries (ECC) and the Global Context

ECC constitutes a significant global health issue, affecting hundreds of millions of children. A comprehensive review of ECC highlights the complex interplay of biological, behavioural, socio-economic, and policy factors that contribute to its prevalence (23). Risk factors include prolonged bottle feeding, frequent consumption of sweetened beverages, inadequate fluoride

exposure, and limited access to dental care (23). The societal burden of ECC is substantial: untreated caries potentially leads to pain, infection, malnutrition, speech difficulties, and poor academic performance. Treatment often necessitates general anaesthesia, which carries its own risks and costs.

Children with autism may be at heightened risk of ECC owing to dietary preferences, difficulties in tolerating dental procedures, and medication side effects that reduce salivary flow (13,15,20). Parental stress and competing health priorities potentially delay preventive visits (17). Nevertheless, the evidence remains mixed, indicating a need for studies that consider the unique social and medical contexts of autistic families (13,15). Our research addresses this gap by focusing on preschool-aged children and analysing both clinical outcomes and biological markers.

1.3. Barriers to Dental Healthcare

Accessing dental care is often more complicated for individuals with autism than for their neurotypical peers (17,33). Sensory hypersensitivity means that the sights, sounds, and tactile sensations of a dental clinic can provoke anxiety and lead to avoidance (6,17). High-pitched noises, bright lights, unfamiliar tastes and smells, and close physical contact potentially trigger distress and hinder cooperation. Consequently, numerous families postpone routine check-ups and seek care only when pain or infection arises, thereby increasing the likelihood of invasive treatments (13,17,33).

Communication differences further complicate access to dental care for children with autism. These children may exhibit limited verbal expression or rely on alternative communication methods, rendering it difficult for them to describe pain or follow instructions. They may also experience sensory sensitivity and behavioural challenges that interfere with cooperation during dental procedures (33). Dental professionals may misinterpret behaviours as non-compliance rather than as responses to anxiety or sensory overload. Even when dentists exhibit sympathy, a lack of specialised training in behavioural strategies can undermine effective care. Some practitioners may default to sedation or general anaesthesia rather than employing desensitisation and gradual exposure techniques. Although sedation is occasionally necessary, an over-reliance on it may diminish opportunities for children to acclimatise to dental procedures and develop coping strategies.

Socio-economic factors and service structures further complicate access. Families may struggle with the costs associated with specialised dental care, particularly when appointments are

prolonged and demand additional resources. Insurance schemes may not cover behavioural support or the extra time required. Geographical disparities potentially limit access to clinics accommodating patients with autism, particularly for families in rural or low-income areas. Parental stress and competing healthcare appointments may also diminish the priority assigned to dental visits. Culturally appropriate educational materials and flexible scheduling are essential to support diverse families (17,34).

Health system barriers include lengthy waiting lists for specialist services, a lack of integration between medical and dental care, and policies that fail to recognise the additional time and costs associated with treating patients with autism (5,17,34). Advocacy groups emphasise the need to train dental students and practising clinicians in neurodiversity-affirming approaches. Environmental modifications, such as mitigating sensory stimuli, providing visual schedules, and allowing familiarisation visits, can significantly enhance cooperation and alleviate anxiety (17). Nonetheless, the implementation of these strategies remains inconsistent and is largely reliant on the initiative of individual practitioners (17,33).

1.4. Behavioural Determinants of Oral Health

Oral health behaviours, including the frequency of brushing, the use of fluoridated toothpaste, and dietary habits, play a central role in the prevention of caries and periodontal disease (16). Research indicates that children with autism frequently demonstrate less consistent dental hygiene routines than their neurotypical peers (13,15). A case-control study conducted in Jordan found that individuals with autism brushed less frequently and had lower oral health knowledge scores than controls (19). This study underscored the importance of caregiver education and support in establishing effective routines. Similar findings were reported in a recent scoping review, which summarised the challenges encountered by parents and caregivers in maintaining oral hygiene among children with autism (19). Common obstacles included resistance to tooth brushing owing to sensory aversion, difficulty understanding instructions, and the lack of time and training for caregivers (33).

Behavioural determinants of oral health are influenced by the sensory sensitivities, cognitive styles, and executive functioning differences associated with autism (3,6,12). For certain children, the sensation of bristles or the taste of toothpaste can be overwhelming; for others, difficulties with motor planning render brushing cumbersome. Reward systems, social stories, and modelling can enhance compliance but require individualised adaptation. Occupational

therapists often collaborate with families to develop desensitisation protocols and select adaptive tools, such as toothbrushes with varying textures, or automated timers (20,33,35,36).

Dietary patterns reflect behavioural determinants in children with autism (12,13). Many of these children exhibit a preference for carbohydrate-rich or soft foods, often avoiding fruits and vegetables owing to texture or taste (13,37). They may graze throughout the day instead of adhering to structured meal times, resulting in frequent acid attacks on their teeth (16). Food selectivity can also lead to nutritional imbalances that adversely affect enamel development. Interventions aimed at promoting the acceptance of a broader range of foods potentially benefit both oral and general health (36,37).

Sleep patterns and stress levels also significantly influence behaviour. Sleep disturbances, which are common in individuals with ASD, can disrupt morning routines, including tooth brushing, and may contribute to bruxism and other parafunctional habits (21,22). Addressing issues related to sleep hygiene and stress management may indirectly enhance oral health behaviours (21,22). Considering the diversity of presentations in ASD, behavioural strategies must be personalised and family centred (6).

1.5. Caregiver Perspectives and Quality of Life

Caregivers play a crucial role in the oral health of children with autism (17,33). Factors such as parental stress, competing demands, and a lack of confidence can adversely affect oral hygiene practices (33). Qualitative studies indicate that caregivers often feel overwhelmed by the multitude of medical and therapy appointments, leading to the deprioritization of dental care. They may perceive dental procedures as traumatic for their child and thus avoid regular visits until absolutely necessary (17,34). Educational programmes that empower caregivers with behavioural management skills and oral hygiene techniques potentially lead to improved outcomes (17,33).

Quality of life measures provide insight into the broader impact of oral conditions beyond clinical indices (38). The Parental–Caregiver Perceptions Questionnaire (P-CPQ) and the Child Oral Health Impact Profile (COHIP) are validated tools used to assess oral health-related quality of life (OHRQoL) in paediatric populations (38). In autistic cohorts, higher decayed–missing–filled teeth (dmft/DMFT) scores correlate with poorer P-CPQ scores, reflecting negative impacts on comfort, self-esteem, social participation, and emotional well-being (13,15). Certain studies suggest that children with autism experience more significant negative effects from dental conditions, even when the clinical scores are comparable to those of neurotypical peers

(37,39,40). This may be attributed to heightened sensory sensitivity, communication difficulties, and vulnerability to bullying (15).

Self-reporting is preferred when children can reliably articulate their experiences. However, for younger children and those with significant communication challenges, caregiver reports serve as proxies. The reliability of caregiver reporting potentially varies in the light of their own stress levels and understanding of the child's experiences (33,40). Nonetheless, caregiver perceptions remain valuable for identifying areas requiring support and tailoring interventions (33). Improving oral health may enhance the overall quality of life of autistic children, alleviating pain, improving nutrition, and facilitating social participation (15,40).

1.6. Standardised Assessment Frameworks

Accurate assessments of oral health in children with autism necessitate the use of standardised tools and protocols (23,41). The World Health Organization's Oral Health Surveys provide a framework for the consistent collection of data across populations (41). These surveys recommend clinical examinations of caries, periodontal status, dental erosion, and oral hygiene, supplemented by questionnaires on dietary habits and access to care (41). In research settings, indices such as the dmft/DMFT Index, Gingival Index (GI), Plaque Index (PI), and Basic Erosive Wear Examination (BEWE) are commonly employed to quantify disease burden (23,41).

For quality-of-life assessments, instruments such as the P-CPQ and Paediatric Quality of Life Inventory™ (PedsQL™) have been validated for use in both neurotypical and autistic populations (42). The Parental-Caregiver Perceptions Questionnaire (P-CPQ) has been used to evaluate oral health–related quality of life (38). These tools assist researchers and clinicians in understanding the intersection of oral health with emotional, social, and functional domains, thereby guiding holistic care (40,42).

1.7. Microbial Ecology and Cross-kingdom Interactions

The oral cavity harbours a complex microbial community that begins to form at birth and evolves during early childhood. Longitudinal studies indicate that the oral microbiome undergoes ecological succession influenced by delivery mode, feeding practices, antibiotic exposure, and environmental factors (43). By approximately 2 years of age, the microbial community approaches an adult-like state, although it remains more susceptible to perturbation (43). Early colonisation by cariogenic streptococci sets the stage for subsequent biofilm

composition (23). Reduced microbial diversity and the dominance of acidogenic species are hallmarks of the dysbiosis associated with caries (16,23).

Recent research has underscored the role of fungi in oral ecology. *Candida albicans*, a common commensal organism, can form pathogenic biofilms in the presence of sugars and compromised immune function (25,29,32). In mixed biofilms with *S. mutans*, *C. albicans* contributes to extracellular matrix production and acid stability, thereby potentiating enamel demineralisation (25,26). This synergy emanates from physical interactions where *C. albicans* mannans bind to *S. mutans* glucosyltransferase B and metabolic cooperation, in which fungal metabolites support bacterial growth (27,28). *In vivo* studies have demonstrated that these cross-kingdom biofilms exhibit greater resistance to environmental insults and antimicrobial agents than single-species biofilms (25). Notably, diet and saliva modulate biofilm formation, sucrose consumption enhances cross-kingdom interactions, while salivary proteins can either inhibit or promote adhesion, depending on their composition (29).

C. albicans overgrowth and altered bacterial communities have been observed in children with autism, although the direction of causality remains unclear (24,31). Some hypotheses suggest that immune dysregulation and gastrointestinal issues common in autism predispose individuals to fungal colonisation, which may, in turn, influence systemic inflammation and behaviour (30). Others propose that dietary patterns and oral hygiene practices drive dysbiosis independently of neurodevelopmental diagnoses (24,25). Continued research into the oral microbiome of children with autism could potentially reveal biomarkers for the early identification of caries risk and targets for probiotics or dietary interventions (26,32). These findings indicate that fungal colonisation may represent an additional biological factor influencing oral health in children with ASD (25,27).

1.8. Neuroendocrine Dysregulation

Beyond microbial and behavioural factors, hormonal and neuroimmune systems also influence oral health (30). Individuals with autism frequently exhibit alterations in the hypothalamic–pituitary–adrenal axis, as evidenced by atypical cortisol rhythms and stress responses (44). Reviews of cortisol responsiveness indicate that some children with autism demonstrate blunted diurnal variation, characterised by lower morning peaks and higher evening levels, while others exhibit heightened stress reactivity (44). These patterns may be associated with anxiety, behavioural regulation, and sensory sensitivities. Research exploring salivary steroid hormones

identified differences in cortisol and androgen metabolites between autistic and control boys (45).

Melatonin, a hormone that regulates circadian rhythms, is often found to be reduced or phase-shifted in children with autism. A comprehensive review of melatonin research revealed that numerous individuals with autism experience delayed melatonin onset, reduced nighttime secretion, and lower total melatonin levels (46). Clinical trials involving melatonin supplementation have shown improvements in sleep onset and duration among children with autism; however, the effects on behaviour are variable. A recent scoping review underlined the potential of salivary biomarkers, including melatonin and cortisol, for the early detection of autism, and for monitoring interventions (32). Advancements in analytical techniques, such as liquid chromatography–mass spectrometry, enable the simultaneous measurement of multiple steroid hormones in saliva or hair, thus providing insights into chronic stress exposure (30).

Factors such as pubertal development, age, and symptom profiles can influence hormone levels and rhythms. One study indicates (27) that cortisol patterns vary throughout childhood and adolescence in individuals with ASD, with puberty affecting diurnal variation. Salivary cortisol responses to stress have been associated with behavioural distress in children with autism, underscoring the relationships among stress, endocrine responses, and oral health experiences (27,28). Neuroendocrine dysregulation may indirectly contribute to oral health by altering immune function, salivary flow, and inflammatory responses. Cortisol is a stress-related hormone that can influence immune responses and physiological regulation, potentially affecting the host's defence against oral pathogens (45). Melatonin possesses antioxidant and anti-inflammatory properties; thus, reduced melatonin levels may impair mucosal protection and tissue repair (46). Inflammation serves as a key mediator, linking neuroendocrine alterations to both neurodevelopmental and oral health outcomes (30). A thorough understanding of these pathways could inform holistic interventions that address stress management, sleep hygiene, and immune support, in conjunction with conventional dental care.

1.9. Biological Vulnerability in Preschool Children

The preschool years represent a critical period of rapid growth and development, during which nutritional status, sleep patterns, and sensory experiences shape health behaviours. Primary teeth begin to erupt at approximately 6 months of age, and by 3 years, the complete set of primary teeth is typically present. These teeth are thinner and less mineralised than permanent

teeth, rendering them more susceptible to caries (16). Healthy primary dentition is essential for activities such as eating, speaking, and social smiling. Consequently, early childhood is a pivotal window for establishing oral hygiene routines and preventive practices (23).

Children on the autism spectrum encounter additional vulnerabilities during this developmental stage (3). Feeding difficulties may lead to nutritional deficiencies that adversely affect tooth development and immune function (12). Sensory hypersensitivities potentially disrupt sleep, resulting in hormonal imbalances that influence appetite and stress responses (21). Many preschoolers with autism exhibit food selectivity, often preferring soft or pureed foods, while rejecting crunchy fruits and vegetables (12). Such dietary habits decrease chewing activity, which typically stimulates saliva flow and aids in clearing food debris (16). Frequent snacking prolongs exposure to fermentable carbohydrates, sustaining acid production (23). When tooth brushing is inconsistent owing to behavioural resistance, plaque accumulation occurs, accelerating demineralisation (36). Additionally, recurrent antibiotic treatments for ear or respiratory infections may disrupt the oral microbiome, potentially favouring opportunistic organisms such as Candida, which has been associated with bacterial community changes in children with severe early childhood caries (43,47).

Parental awareness and support are crucial for mitigating the vulnerabilities related to oral health (19,33). Educating caregivers regarding tooth eruption patterns, the importance of fluoride, and dietary modifications, can empower them to intervene early (16,23). Paediatricians, therapists, and dental professionals should collaborate to screen for oral health issues during routine visits, and provide anticipatory guidance (17,34). Research focusing on preschool populations is crucial, as early intervention can prevent progression to severe caries and periodontal disease (23). Longitudinal studies are necessary to track how early behaviours and biological markers predict later outcomes in both autistic and neurotypical children (2,5).

1.10. Research Gaps

Despite a growing interest in the oral health of individuals with autism, several gaps persist. Numerous studies employ cross-sectional designs and rely on convenience samples, limiting the generalisability of findings. Sample sizes are often small, and methodological heterogeneity, including variations in diagnostic criteria, age ranges, caries indices, and control groups, complicates comparisons across studies (13-15,18-21,39). Meta-analyses reveal considerable variability in reported caries prevalence and periodontal outcomes, suggesting that unmeasured

confounders, such as diet, access to care, and socio-economic status, may contribute to these mixed findings (13-15,39).

Another gap exists in the integration of biological markers with clinical and behavioural data. Although research has identified dysregulated cortisol and melatonin rhythms in autistic individuals (44-46), few studies have explored how these hormonal patterns correlate with oral health outcomes. Similarly, while *Candida* and bacterial dysbiosis have been associated with severe caries in neurotypical children (25,32), the prevalence and impact of these cross-kingdom interactions in children with autism remain largely underexplored. Most microbiome studies have failed to consider endocrine factors or the behavioural context, hindering the interpretation of causality.

The preschool period is underrepresented in the literature. Multiple investigations have focused on school-aged children or adolescents, despite the fact that the foundations of oral health are established earlier. The interplay of emerging self-care behaviours, changing microbiota, and developing neuroendocrine systems may present unique risks or protective factors for young children with autism.

Finally, caregiver perspectives and quality of life measures are not consistently incorporated into clinical studies. Without an understanding of how oral conditions affect daily functioning and family well-being, interventions may overlook critical outcomes. Interdisciplinary research that combines clinical assessment, laboratory measures, and psychosocial evaluation is imperative.

Our study addresses these gaps by examining salivary cortisol and melatonin levels in relation to oral health parameters and *C. albicans* colonisation in preschool children, with and without ASD. By incorporating behavioural questionnaires and caregiver-reported quality of life measures, we aim to provide a comprehensive understanding of the factors influencing oral health in this population.

A comprehensive approach that integrates clinical assessment, hormonal and microbial analyses, and caregiver perspectives is essential to unravel these complex relationships. By elucidating the mechanisms by which endocrine factors, microbial ecology, and behaviours intersect to influence oral health in individuals with autism, personalised strategies could be developed. These could prevent disease, mitigate disparities, and improve the quality of life of children with autism, and their families.

This integrative approach is indispensable, as comprehending the interactions among endocrine regulation, microbial ecology, and behavioural determinants may facilitate early identification and the formulation of personalised preventive strategies in paediatric dentistry.

2. AIMS AND HYPOTHESES

2.1. Objectives

General objective

This doctoral dissertation aims to examine the relationship between hormone levels, cortisol and melatonin, and oral health in children with Autism Spectrum Disorder, ASD, compared with healthy children, while identifying potential influencing factors.

Specific objectives

- To determine differences in cortisol and melatonin levels between children with ASD and healthy children.
- To assess variations in dental caries, gingival health, dental erosion, and Candida albicans prevalence between children with ASD and healthy children.
- To identify factors such as sensory sensitivities, communication difficulties, and parental influences that may impact oral hygiene in children with ASD and healthy children.

2.2. Hypothesis

Children with Autism Spectrum Disorder (ASD) have poorer oral health outcomes, with a higher prevalence of dental caries, gingival inflammation, dental erosion, and Candida albicans colonization, associated with elevated cortisol levels, reduced melatonin levels, and challenges in sensory processing and communication, compared with healthy children.

3. PARTICIPANTS AND METHODS

Ethical approval for this study was obtained from the Municipality of Pristina under the municipal health and safety regulations (Ref. No. 03-615/03-514). This work was also ethically reviewed and approved by the Dental Chamber of Kosovo (approval code: 49/24.05.2024), as well as by the University of Zagreb's Ethics Committee (file number: 05-PA-6 29/2024). Before the start of any procedure, written informed consent was obtained from the parents or guardians. This work was performed in accordance with the principles of the Declaration of Helsinki (48).

3.1. Participants

A cross-sectional study with a comparative design was conducted. Participants with ASD were recruited from children aged 4-6 attending consultations at the Centre for Rehabilitation and Education, the Clinic of Clinical Psychology Specialists, and the Institute of Psychological Services in Pristina, where this research was also conducted. As a control group, healthy children were recruited from public preschool institutions in Pristina, with their healthy status confirmed by surveying the preschool enrolment database. The same protocol and assessment tools were used in both groups. Before a scheduled visit to one of the above-mentioned institutions, the parents or guardians of the children were contacted by phone and asked if they would like their children to participate in the study.

The necessary sample size was determined utilizing the G*Power 3.1 application (49). By incorporating initial data and assuming the medium effect size (Cohen's $d = 0.6$), along with the designated significance level ($\alpha = 0.05$) and statistical power ($1–\beta = 0.80$), the minimum sample size was estimated to be 72 participants. In order to enhance statistical robustness and accommodate for attrition, a sample of 90 participants was selected for the research, comprising 45 individuals diagnosed with ASD and an equal number in the control group.

To be eligible for inclusion in the ASD group, a documented ASD diagnosis in the medical records was required, based on DSM-5-TR criteria (50). Inclusion criteria were as follows: age 4-6 years; ASD diagnosis without intellectual disability (ASD group only); no systemic diseases. Exclusion criteria were as follows: epilepsy or other neurological conditions; genetic syndromes associated with autism (e.g., fragile X, Rett); IQ < 70; current antifungal or antibiotic therapy; systemic hormonal therapy; and endocrine disorders such as hypothyroidism.

The sample consisted of 90 children aged 4-6 years. Forty-five children had a diagnosis of ASD without intellectual disability, and the other 45 were neurotypical controls. Recruitment of the two groups occurred simultaneously to minimize temporal bias, and the groups were sex

matched. Children with ASD were identified through the participating clinics and required a pre-existing ASD diagnosis with full-scale IQ ≥ 70, as confirmed using the Vineland Adaptive Behavior Scales (51) and standardized intelligence tests (e.g., Wechsler Preschool and Primary Scale of Intelligence or Raven's Progressive Matrices) (52). Control participants were recruited from public preschools in Pristina; their health status was confirmed via official preschool registration records, including vaccination history, developmental assessments, and medical history.

3.2. Methods

Oral health status was assessed by a single calibrated examiner using the WHO's Oral Health Surveys – Basic Methods (5th edition) (53). Two weeks before the examination, flashcards showing a dentist holding a dental mirror and a child undergoing an oral examination were given to the children. This series of cards helped children on the autism spectrum to become familiar with the setting of their upcoming dental visit and feel more relaxed about it. Parents were asked to tell the story using the cards and show the pictures to their children at least three times a day for a week. During the visit, the oral health status of each participant was examined while they were seated in a dental chair, under natural light using an intraoral examination mirror and a WHO probe, with appropriate personal protective equipment. If necessary, a mouth-opener was used.

3.2.1. Oral Health Assessment

Four indices were used to assess oral health. The first was the dmft Index. The scores of this index were dichotomized to represent the presence of dental caries (dmft ≥ 1) and its absence (dmft = 0). The GI (Gingival Index) was used to assess gingival status and to record clinical variations in the gingiva (54). Considering the age of participants, and especially the ASD group, this examination was adjusted to only evaluate the overall gingival status visually; to reduce anxiety among the participants, bleeding upon probing of selected index teeth was not examined. The colour, shape, size, and structure of the gingiva were observed and scored. Gingiva that had no clinical changes in these components was scored as "0" and categorized as normal. Gingiva that had slight clinical changes in colour, shape, size, and structure was scored as "1" and categorized as being mildly inflamed. Gingiva that had changes in colour, size, and shape accompanied by visible signs of bleeding, was scored as "2" and categorized as being moderately inflamed. Gingiva that was swollen and exhibited spontaneous bleeding was scored as "3" and categorized as being severely inflamed. Similar adapted assessments were employed

in a study in Chennai to make the procedure less challenging for those with ASD (55). Those researchers confirmed that there was no evidence that these adaptations compromised the validity of the results. The third index used for assessing oral health here was the PI (Plaque Index), also known as the Hygiene Index. In this study, the examination to determine this index was conducted in a dental chair using a dental mirror and a WHO probe, with the focus on the presence and quantity of plaque on selected teeth. The PI scores were grouped into three categories: good (scores 0 to 1), fair (scores 1 to 2), and poor (scores 2 to 3), enabling the comparison of oral hygiene levels between the study groups. Finally, the BEWE (Basic Erosive Wear Examination) was performed to evaluate erosive tooth wear in accordance with the guidelines presented by Bartlett and Ganss (56). The highest score for each sextant was determined by the extent of wear observed on its individual teeth, with a scale including the numbers 0 (indicating no erosion), 1 (representing initial surface texture loss), 2 (denoting a distinct defect with less than 50 % hard tissue loss), and 3 (indicating hard tissue loss of 50 % or more). Each subject's cumulative score was calculated by summing their sextant scores, with risk levels classified as follows: none (0–2), low (3–8), moderate (9–13), and high (≥ 14).

3.2.2. Amalgamated WHO Oral Health Questionnaire

In addition to the clinical oral examination, parents/guardians completed an adapted version of the WHO Oral Health Questionnaire (53) to assess oral health behaviours in the children. Besides the standard questionnaire, we included additional questions relevant to the ASD group to collect information on self-injurious behaviours and medication use.

The standard WHO Oral Health Questionnaire for Children/Adolescents (items 1–15) was independently translated from English into Albanian by two translators. The two translations were then harmonized and back-translated by a third translator. An expert committee then reviewed discrepancies between the original questionnaire and the back-translation. This led to the addition of 10 items (15–25) to the questionnaire on oral care awareness/behaviour, medication use, and self-injurious behaviours. Content validation was then performed by three or more experts (paedodontists, psychologists, and public health researchers with experience in autism), who rated the clarity, relevance, and coverage of each item (Likert 1–4), which were used to calculate the I-CVI (Item-level Content Validity Index) and the S-CVI/Ave (Scale-level Content Validity Index, Average method). Scores of ≥ 0.78 for I-CVI and ≥ 0.90 for S-CVI/Ave are considered "acceptable" according to the established guidelines (57). Reliability was also assessed with Cronbach's α (Cronbach's alpha) and test–retest coefficient (ICC Intraclass Correlation Coefficient). Cronbach's α ≥ 0.70 and ICC ≥ 0.75 were interpreted as representing

evidence with acceptable levels of internal consistency and robustness (58,59). Prior to the main study, a pilot test was conducted with parents/guardians (n = 10), who confirmed that they understood the questionnaire well and required only minimal clarification of the wording.

Most items received high ratings for clarity and relevance, with a rating of 1.00 for items Q15–18 and Q24. Items Q19–21, Q22, Q23, and Q25 scored lower (I-CVI = 0.33–0.67), The S-CVI/Ave was 0.70. A Cronbach's α of 0.32 for items Q15–25 indicated poor internal consistency. Test–retest reliability showed consistent responses, with intraclass correlation values meeting the expected standards. Finally, the pilot test (n = 10) provided feedback that confirmed that the questionnaire could be easily understood by the parents/caregivers.

3.2.3. The Parental–Caregivers Perceptions Questionnaire (P-CPQ-16)

This questionnaire was used to assess OHRQoL (38). The instrument was translated into Albanian and culturally adapted independently by two translators (forward translation), followed by harmonization of the two versions and back-translation by a third translator (60). The P-CPQ-16 contains 16 items in four domains (oral symptoms, functional limitations, emotional well-being, and social well-being), rated on a 0–4-point Likert scale (referring to the last 3 months); the total score ranges from 0–64, with higher values representing a greater negative impact (38). A pilot test (n = 10) was conducted before the main study, confirming high clarity and comprehensibility, with minimal adjustments in the wording needed. No new psychometric validation was conducted, given that the P-CPQ-16 had already been strongly validated.

After obtaining written informed consent from the parents/guardians, each child attended a single consultation, at which a dental examination was initially performed and the findings recorded, following WHO protocols. Immediately thereafter, the parents or guardians completed the questionnaires in a quiet room, with a research assistant available to answer any questions about it that they might have. All data were recorded anonymously and entered into a secure database.

A pilot feasibility study was conducted one week before data collection with five preschool children who were not included in the main sample. The pilot assessed the clarity of instructions and the feasibility of saliva collection procedures. The pilot test confirmed full compliance and collection of adequate saliva volume.

3.2.4. Fungal and Hormonal Sampling

Buccal mucosa samples were obtained using sterile transport swabs (61). The swabs were inoculated onto Sabouraud Dextrose Agar supplemented with chloramphenicol (0.05 g/L) and gentamicin (0.02 g/L) to inhibit bacterial growth. Plates were incubated aerobically at 37 °C for 24–72 h. Colonies morphologically consistent with Candida albicans were enumerated, and colony-forming units per millilitre (CFU/mL) were calculated for each sample. Species identification was confirmed using chromogenic and/or biochemical media (CHROMagar, Paris, France).

Unstimulated saliva samples were collected between 20:00 and 21:00 h using Salivette® devices (62). Children refrained from food, beverages (except water), and oral hygiene products for at least 30 min prior to sampling. After a water rinse, an absorbent swab was placed passively under the tongue or between the cheek and gum for 1–2 min. Samples were stored immediately at −20 to −80 °C. In the laboratory, the samples were thawed, centrifuged, and analysed for cortisol and melatonin using competitive ELISA kits (DRG Instruments GmbH, Marburg, Germany) (63). Hormone concentrations were expressed in ng/mL (cortisol) and pg/mL (melatonin).

3.2.5. Statistical Analysis

Data analysis was performed in the statistical programs Statistica 7.1 for Windows and SPSS Statistics 23.0. The following methods were applied:

In the analysis of the series with attribute features (gender, oral presence of Candida, questions from the Oral Health Questionnaire for Children) percentages of structure (%) were determined;

- Differences in the series with attribute features between the ASD group and the control group were analysed using the Pearson Chi-square Test (p), Fisher's Exact Test (p), Pearson Chi-Square / Monte Carlo Sig. (2-sided) / (p), Fisher's Exact Test / Monte Carlo Sig. (2-sided) / (p);
- Descriptive Statistics were prepared (Mean; Std. Deviation; ±95.00 % CI; Median; Minimum; Maximum) for the series with numerical features (years, plaque index, gingival index, dmft index, BEWE index, cortisol, melatonin, the composite values of: oral symptoms, functional limitation, emotional well-being, and social well-being, and total composite scores (P-CPQ-16));
- The distribution of the data was tested with the: Kolmogorov–Smirnov test; Lilliefors test; Shapiro–Wilks test (p).

- The differences between the ASD group and the control group were tested with the Mann–Whitney U test (Z/p);
- In the segment on oral symptoms, functional limitation, emotional well-being, and social well-being / (P-CPQ-16), internal consistency between the responses was analysed using Reliability Statistics / Cronbach's Alpha;
- Correlations within the following relationships were analysed using Spearman Rank Order R (R/p): dmft index and Candida albicans (CFU/mL); Gingival Index (GI) and Candida albicans (CFU/mL); Plaque Index (PI) and Candida albicans (CFU/mL); BEWE Index and Candida albicans (CFU/mL); dmft index and Cortisol ng/mL; Gingival Index (GI) and Cortisol ng/mL; Plaque Index (PI) and Cortisol ng/mL; BEWE Index and Cortisol ng/mL; dmft index and Melatonin pg/mL; Gingival Index (GI) and Melatonin pg/mL; Plaque Index (PI) and Melatonin pg/mL; BEWE Index and Melatonin pg/mL; Cortisol ng/mL and Melatonin pg/mL; Gingival Index (GI) and dmft index; BEWE index and Plaque Index (PI);
- Differences in the Spearman rank-order correlation coefficients (ρ) between the ASD group and the control group were evaluated using the Fisher r-to-z transformation (Excel 2010). Correlation analyses between cortisol concentrations (ng/mL) and Candida albicans counts (CFU/mL), as well as between melatonin concentrations (pg/mL) and Candida albicans counts (CFU/mL), were conducted separately for the ASD and control groups using Spearman rank-order correlation analysis. Correlation coefficients (ρ) and the corresponding p values were reported;
- Correlations were analysed using Multiple Regression / R /p in the following relationships: dmft index dependent variable and sex (boy = 1; girl = 0), age, PI, CA (Candida albicans / 1 = positive; 0 = negative), CA(CFU) / number of colonies, independent variables; Cortisol dependent variable and Melatonin, Gingival index (GI), CA (Candida albicans / 1 = positive; 0 = negative), age, independent variables; the BEWE index is a dependent variable, and the Age, Plaque Index (PI), dmft index, Cortisol are independent variables;
- When determining the predictive values of Sex (boy = 1; girl = 0), Age, dmft index, Cortisol, Melatonin for the presence of CA (Candida albicans), the Binary Logistic Regression / (Wald, Exp (B) / 95.0 % CI for Exp (B)/(p) / method was applied.
- Significance was determined at $p < 0.05$. The data are presented in tables and graphs.

4. RESULTS

4.1. Characteristics of the study sample

4.1.1. Sociodemographic characteristics

The study included a group of 90 children aged 4–6 years, divided into two groups: 45 children with Autism Spectrum Disorder (ASD) and 45 children in the control group. The distribution of children by sex is presented in Table 1 and Figure 1. In the control group, 23(51.1%) were male, and 22(48.9%) were female. The same distribution was observed in the ASD group, where 23 children (51.1%) were male and 22 (48.9%) were female.

For Pearson Chi-Square = 0.000 and p>0.05(p=1.000) / Asymp.Sig(2-sided), there is no significant difference in terms of the gender of children between the two groups.

Table 1. Distribution of children by group and sex

			Sex		Total
			Boy	Girl	
Group	Control	Count	23	22	45
		%	51,1%	48,9%	100,0%
	ASD	Count	23	22	45
		%	51,1%	48,9%	100,0%
	Total	Count	46	44	90
		%	51,1%	48,9%	100,0%

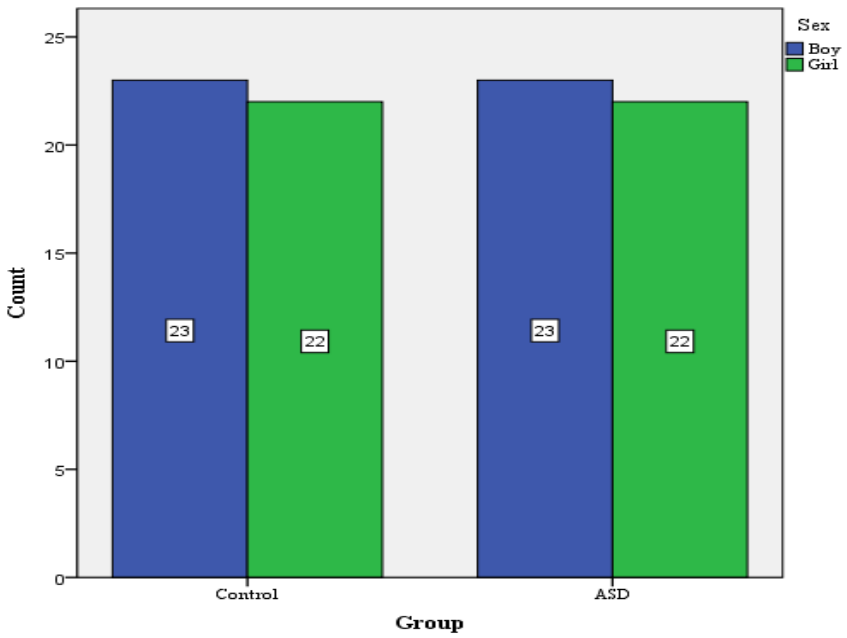

Figure 1. Percentage distribution of children by group and sex

The distribution of children according to place of residence is presented in Figure 2. Of the 45 children in the control group, 41 (91.1%) lived in urban areas, 1 (2.2%) lived in peri-urban areas, and 3 (6.7%) lived in rural areas.

Of the 45 children in the ASD group, 28(62.2%) lived in urban, 4(8.9%) lived in periurban, and 13(28.9%) lived in rural areas.

For Fisher's Exact Test = 10.382 and p<0.01(p=0.005) / Monte Carlo Sig.(2-sided) / 0.003-0.006 / there is a significant difference in the place of residence of the children in both groups, as shown in Figure 2.

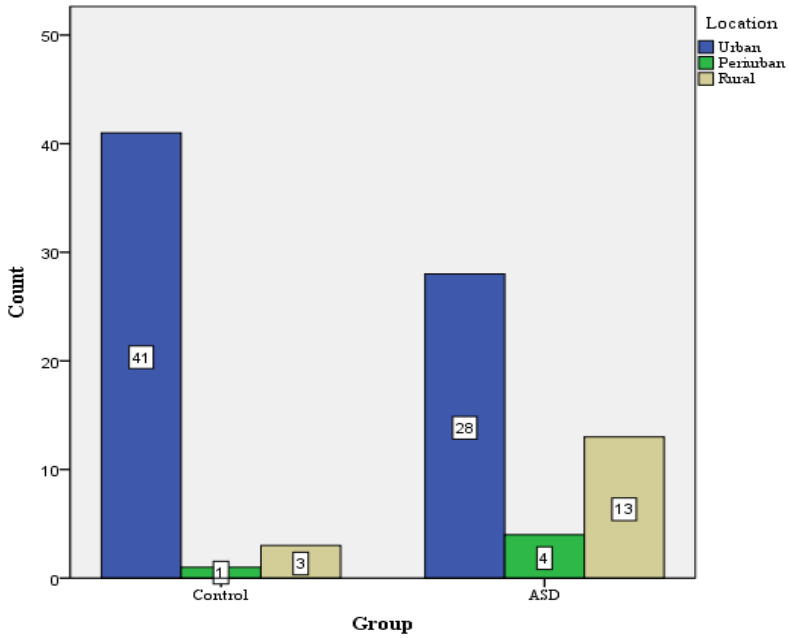

Figure 2. Percentage distribution of children by group and place of residence

In addition to the children's sociodemographic characteristics, family-related factors were also evaluated. Parental education was analyzed because it may influence children's oral hygiene habits and access to oral health care. Therefore, the educational levels of fathers and mothers were assessed.The father's level of education is presented in Table 2 and Figure 3.

The father's level of education is presented in Table 34 and Figure 25. In the control group, the highest proportion of fathers had completed college or university, 26 fathers, 57.8%, followed by high school, 17 fathers, 37.8%. Only 2 fathers, 4.4%, had completed secondary school.

In the ASD group, 19 fathers, 42.2%, had completed college or university, followed by secondary school, 14 fathers, 31.1%, and high school, 9 fathers, 20.0%. Primary school

education was reported for 3 fathers, 6.7%.For Fisher's Exact Test = 15.489 and p<0.01(p=0.001) / Monte Carlo Sig.(2-sided) / 0.000-0.002 / there is a significant difference in the children's responses between the two groups.

Table 2. Fathers' educational level by study group

			What level of education has your father completed? (Q12)				Total
			Primary school completed	Secondary school completed	High school completed	College / university completed	
Group	Control	Count	0	2	17	26	45
		%	0,0%	4,4%	37,8%	57,8%	100,0%
	ASD	Count	3	14	9	19	45
		%	6,7%	31,1%	20,0%	42,2%	100,0%
Total		Count	3	16	26	45	90
		%	3,3%	17,8%	28,9%	50,0%	100,0%

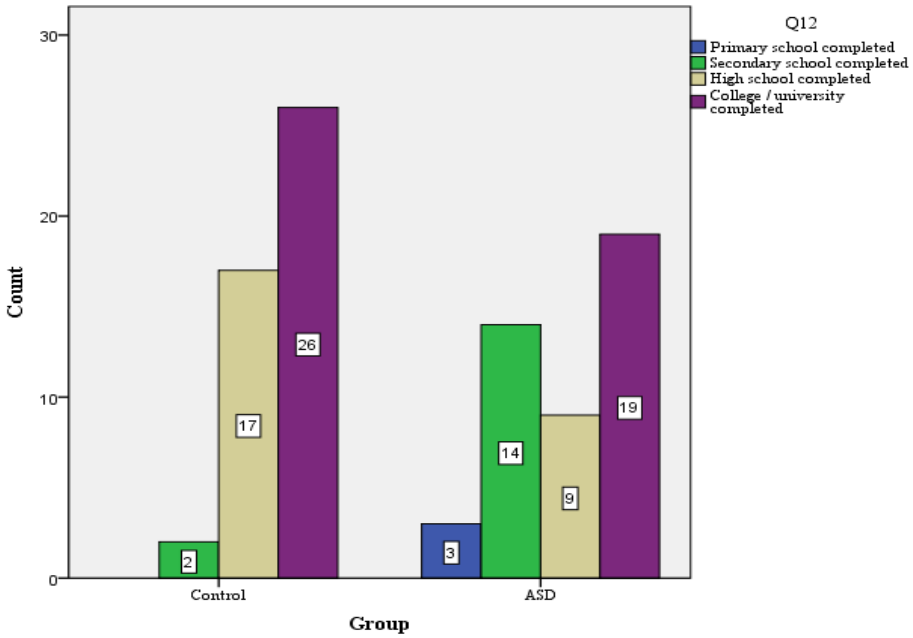

Figure 3. Percentage distribution of father's level of education by group

The mother's level of education was also analyzed as a family-related factor and is presented in Table 3 and Figure 4. In the control group, the highest percentage of mothers had completed college or university, 26 mothers, 57.8%, followed by high school, 16 mothers, 35.6%. In the ASD group, 21 mothers, 46.7%, had completed college or university, while 12 mothers, 26.7%, had completed secondary school.

Table 3. Mothers' educational level by study group

			What level of education has your mother completed? (Q13)				Total
			Primary school completed	Secondary school completed	High school completed	College / university completed	
Group	Control	Count	1	2	16	26	45
		%	2,2%	4,4%	35,6%	57,8%	100,0%
	ASD	Count	5	12	7	21	45
		%	11,1%	26,7%	15,6%	46,7%	100,0%
Total		Count	6	14	23	47	90
		%	6,7%	15,6%	25,6%	52,2%	100,0%

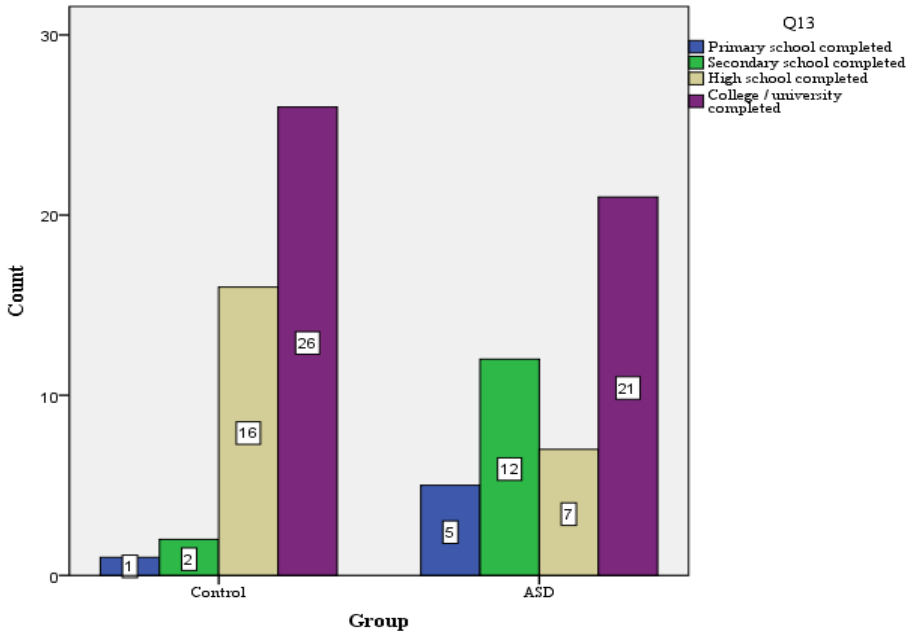

Figure 4. Percentage distribution of mother's level of education by group

4.1.2. Oral hygiene habits

After describing the sociodemographic characteristics, oral hygiene habits were analyzed. The frequency of tooth brushing among children in both groups is presented in Table 4 and Figure 5. Of the 45 children in the control group, 7(15.6%) brushed their teeth several times a month (2–3 times), 2(4.4%) brushed their teeth once a week, 8(17.8%) brushed their teeth several times a week (2–6 times), 16(35.6%) brushed their teeth once a day and 12(26.7%) brushed their teeth 2 or more times a day.

Of the 45 children in the ASD group, 10(22.2%) brushed their teeth several times a month (2–3 times), 2(4.4%) brushed their teeth once a week, 19(42.2%) brushed their teeth several times a week (2–6 times), 9(20.0%) brushed their teeth once a day and 5(11.1%) brushed their teeth 2 or more times a day.

For Fisher's Exact Test = 209.854 and $p<0.05$($p=0.035$) / Monte Carlo Sig.(2-sided) / 0.030-0.040 / there is a significant difference in the frequency of brushing teeth in children in both groups.

Table 4. Frequency of tooth brushing among children by group

			Group		Total
			Control	ASD	
Q6	Several times a month (2–3 times)	Count	7	10	17
		%	15,6%	22,2%	18,9%
	Once a week	Count	2	2	4
		%	4,4%	4,4%	4,4%
	Several times a week (2–6 times)	Count	8	19	27
		%	17,8%	42,2%	30,0%
	Once a day	Count	16	9	25
		%	35,6%	20,0%	27,8%
	2 or more times a day	Count	12	5	17
		%	26,7%	11,1%	18,9%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

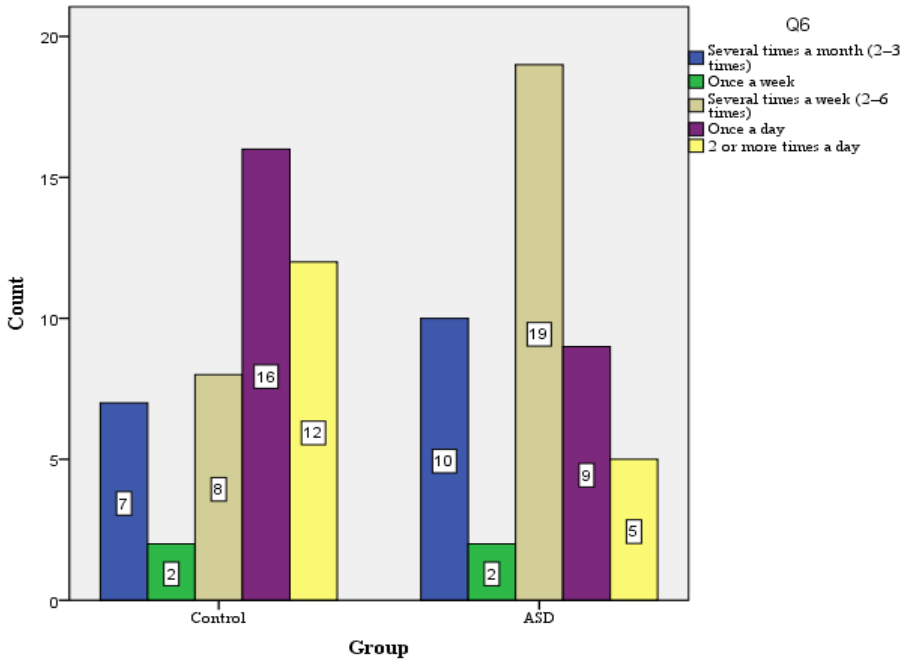

Figure 5. Percentage distribution of tooth brushing frequency by group

For Fisher's Exact Test = 1.715 and p>0.05 (p=0.581) / Monte Carlo Sig. (2-sided) / 0.568-0.594 / there is no significant difference in the responses of the children between the two groups.

4.1.3. Dietary habits

The dietary habits of children in both groups were analyzed according to the frequency of consumption of different foods and drinks. The results are presented in Tables 5–13 and Figures 6–13.

The frequency of fresh fruit consumption is presented in Table 5 and Figure 6. In the control group, the most common responses were consumption every day, reported by 16 children, 35.6%, and several times a week, reported by 14 children, 31.1%. In the ASD group, the most common response was consumption several times a week, reported by 17 children, 37.8%, followed by every day, reported by 13 children, 28.9%. For Fisher's Exact Test = 7.289 and p>0.05(p=0.167) / Monte Carlo Sig.(2-sided) / 0.158-0.177 / there is no significant difference in the children's responses between the two groups.

Table 5. Frequency of fresh fruit consumption by group

			Group		Total
			Control	ASD	
Q10.1	Never	Count	0	3	3
		%	0,0%	6,7%	3,3%
	Several times a month	Count	2	0	2
		%	4,4%	0,0%	2,2%
	Once a week	Count	1	4	5
		%	2,2%	8,9%	5,6%
	Several times a week	Count	14	17	31
		%	31,1%	37,8%	34,4%
	Every day	Count	16	13	29
		%	35,6%	28,9%	32,2%
	Several times a day	Count	12	8	20
		%	26,7%	17,8%	22,2%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

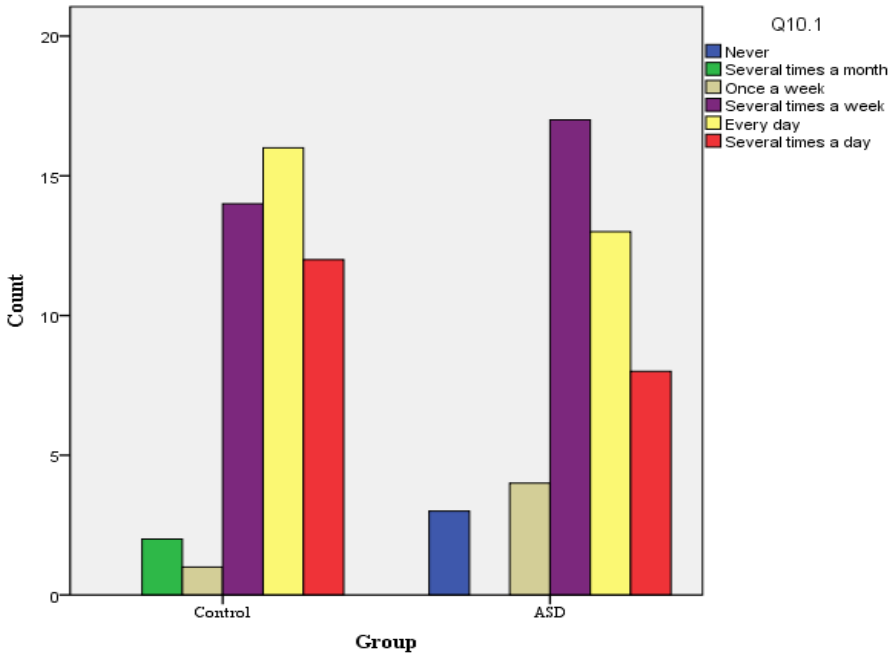

Figure 6. Percentage distribution of fresh fruit consumption by group

The frequency of consumption of biscuits, cakes, pastries, and sweet bread is presented in Table 6 and Figure 7. In both groups, the most common response was consumption several times a week. This was reported by 24 children, 53.3%, in the control group and 17 children, 37.8%, in the ASD group. Daily consumption was reported by 11 children, 24.4%, in the control group and 9 children, 20.0%, in the ASD group. Fisher's Exact Test showed no statistically significant difference between the groups regarding the consumption of biscuits, cakes, pastries, and sweet bread, Fisher's Exact Test = 5.684, p = 0.342.

Table 6. Frequency of biscuits, cakes, pastries, and sweet bread consumption by group

			Group		Total
			Control	ASD	
Q10.2	Never	Count	1	3	4
		%	2,2%	6,7%	4,4%
	Several times a month	Count	4	4	8
		%	8,9%	8,9%	8,9%
	Once a week	Count	4	7	11
		%	8,9%	15,6%	12,2%
	Several times a week	Count	24	17	41
		%	53,3%	37,8%	45,6%
	Every day	Count	11	9	20
		%	24,4%	20,0%	22,2%
	Several times a day	Count	1	5	6
		%	2,2%	11,1%	6,7%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

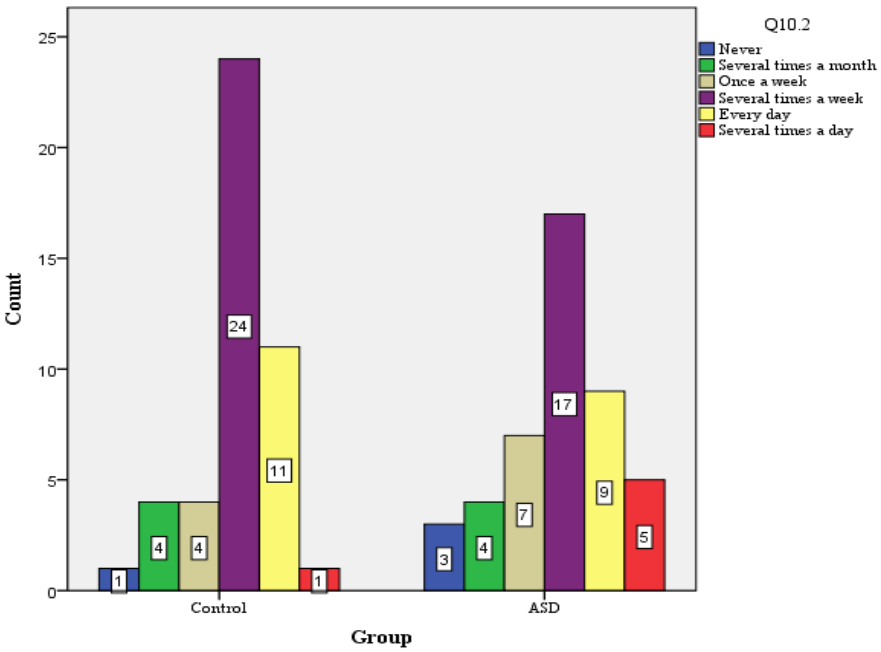

Figure 7. Percentage distribution of biscuits, cakes, pastries, and sweet bread consumption by group

Consumption of lemonade, Coca-Cola, and other sodas is presented in Table 7 and Figure 8. In the control group, the most frequent response was consumption several times a week, reported by 13 children, 28.9%, while 11 children, 24.4%, reported never consuming these drinks or consuming them several times a month. In the ASD group, the most frequent response was never, reported by 16 children, 35.6%, followed by several times a month, reported by 14 children, 31.1%. Fisher's Exact Test showed no significant difference between the groups, p=0.465.

Table 7. Frequency of lemonade, Coca-Cola, and other soda consumption by group

			Group		Total
			Control	ASD	
Q10.3	Never	Count	11	16	27
		%	24,4%	35,6%	30,0%
	Several times a month	Count	11	14	25
		%	24,4%	31,1%	27,8%
	Once a week	Count	8	6	14
		%	17,8%	13,3%	15,6%
	Several times a week	Count	13	6	19
		%	28,9%	13,3%	21,1%
	Every day	Count	1	1	2
		%	2,2%	2,2%	2,2%
	Several times a day	Count	1	2	3
		%	2,2%	4,4%	3,3%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

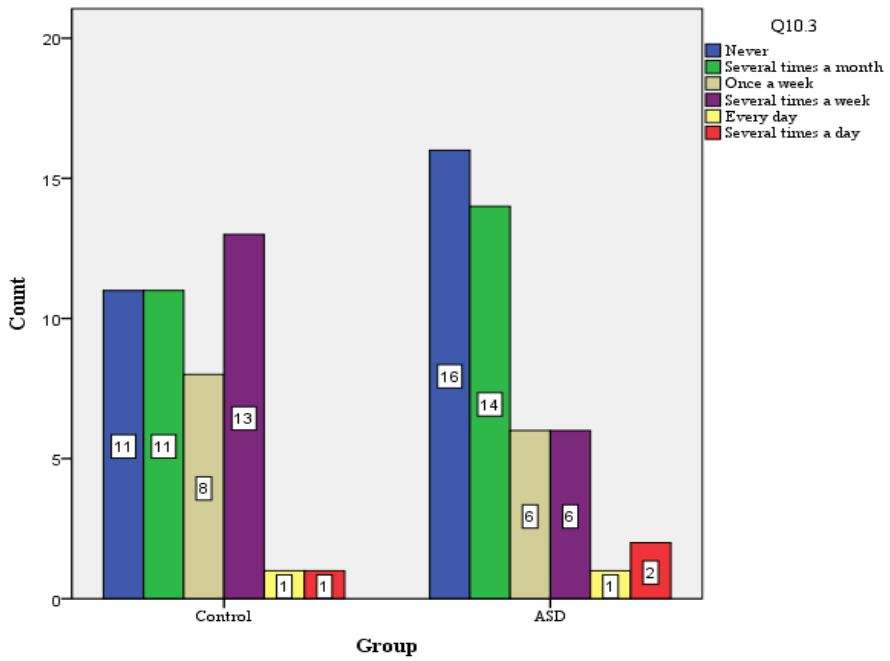

Figure 8. Percentage distribution of lemonade, Coca-Cola, and other soda consumption by group

The frequency of jam and honey consumption is presented in Table 8 and Figure 9. In the control group, the most common response was consumption several times a week, reported by 16 children, 35.6%, followed by never, reported by 15 children, 33.3%. In the ASD group, the most common responses were never and several times a month, each reported by 17 children, 37.8%. Although children in the control group more frequently reported consuming jam and honey several times a week compared with children in the ASD group,

For Fisher's Exact Test = 9.553 and p>0.05(p=0.066) / Monte Carlo Sig.(2-sided) / 0.059-0.072 / there is no significant difference in the children's responses between the two groups.

Table 8. Frequency of jam and honey consumption by group

			Group		Total
			Control	ASD	
Q10.4	Never	Count	15	17	32
		%	33,3%	37,8%	35,6%
	Several times a month	Count	8	17	25
		%	17,8%	37,8%	27,8%
	Once a week	Count	2	2	4
		%	4,4%	4,4%	4,4%
	Several times a week	Count	16	5	21
		%	35,6%	11,1%	23,3%
	Every day	Count	3	3	6
		%	6,7%	6,7%	6,7%
	Several times a day	Count	1	1	2
		%	2,2%	2,2%	2,2%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

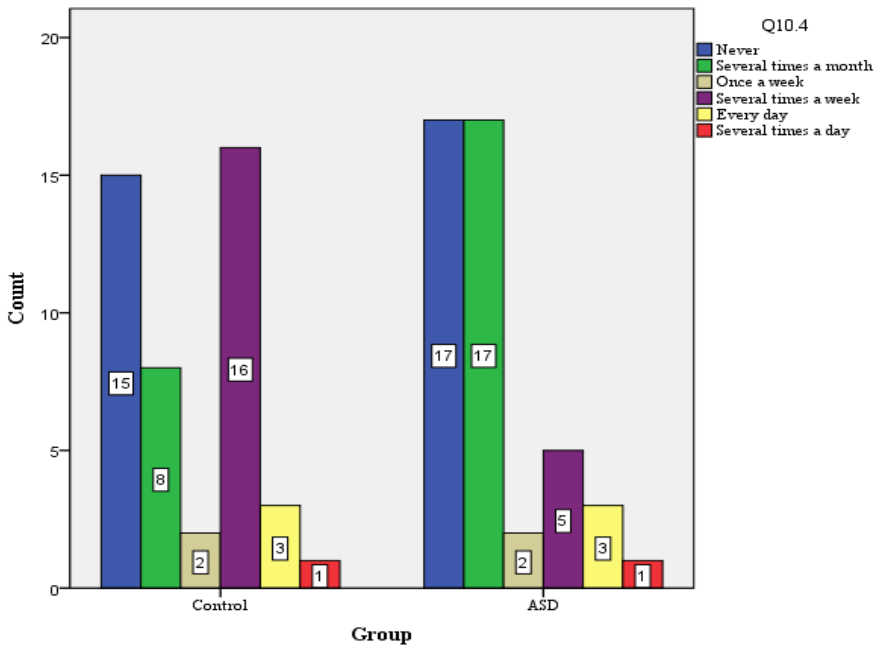

Figure 9. Percentage distribution of jam and honey consumption by group

The frequency of chewing gum with sugar consumption is presented in Table 9 and Figure 10. In the control group, the most common response was consumption several times a month, reported by 16 children, 35.6%, followed by never, reported by 13 children, 28.9%. In the ASD group, more than half of the children, 25 children, 55.6%, reported that they never consumed chewing gum with sugar, while 8 children, 17.8%, consumed it several times a month.

For Fisher's Exact Test = 10.868 and $p<0.05$($p=0.034$) / Monte Carlo Sig.(2-sided) / 0.029-0.072 / there is a significant difference in the children's responses between the two groups.

Table 9. Frequency of chewing gum with sugar consumption by group

			Group		Total
			Control	ASD	
Q10.5	Never	Count	13	25	38
		%	28,9%	55,6%	42,2%
	Several times a month	Count	16	8	24
		%	35,6%	17,8%	26,7%
	Once a week	Count	5	1	6
		%	11,1%	2,2%	6,7%
	Several times a week	Count	8	7	15
		%	17,8%	15,6%	16,7%
	Every day	Count	3	2	5
		%	6,7%	4,4%	5,6%
	Several times a day	Count	0	2	2
		%	0,0%	4,4%	2,2%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

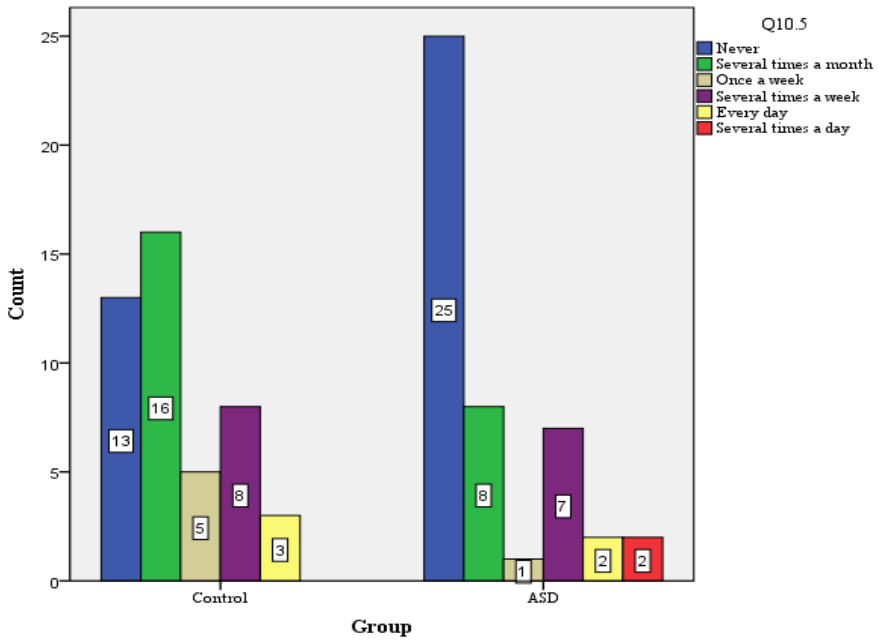

Figure 10. Percentage distribution of chewing gum with sugar consumption by group

The frequency of candies and sweets consumption is presented in Table 10 and Figure 11. In the control group, the most common response was consumption several times a month, reported by 14 children, 31.1%, followed by consumption several times a week, reported by 13 children, 28.9%. In the ASD group, the most common response was consumption several times a week, reported by 13 children, 28.9%, followed by never and several times a month, each reported by 9 children, 20.0%.

Fisher's Exact Test showed no statistically significant difference between the groups regarding candies and sweets consumption, Fisher's Exact Test = 5.258, p = 0.395.Of the 45 children in the ASD group, 9(20.0%) answered never, 9(20.0%) answered several times a month, 8(17.8%) answered once a week, 13(28.9%) answered several times a week, 3(6.7%) answered every day and 3(6.7%) answered several times a day.

For Fisher's Exact Test = 5.258 and p>0.05(p=0.395) / Monte Carlo Sig.(2-sided) / 0.382-0.407 / there is no significant difference in the children's responses between the two groups.

Table 10. Frequency of candy and sweet consumption by group

			Group		Total
			Control	ASD	
Q10.6	Never	Count	8	9	17
		%	17,8%	20,0%	18,9%
	Several times a month	Count	14	9	23
		%	31,1%	20,0%	25,6%
	Once a week	Count	3	8	11
		%	6,7%	17,8%	12,2%
	Several times a week	Count	13	13	26
		%	28,9%	28,9%	28,9%
	Every day	Count	6	3	9
		%	13,3%	6,7%	10,0%
	Several times a day	Count	1	3	4
		%	2,2%	6,7%	4,4%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

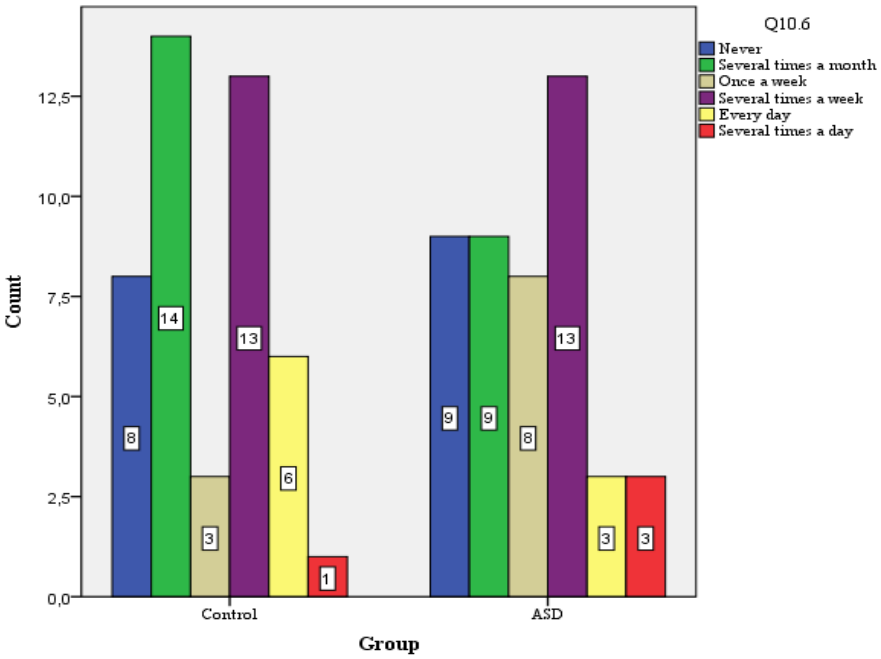

Figure 11. Percentage distribution of candies and sweets consumption by group

The frequency of milk with sugar consumption is presented in Table 11 and Figure 12. In both groups, the majority of children reported that they never consumed milk with sugar, with the same percentage observed in the control and ASD groups, 35 children, 77.8%. Less frequent responses were reported in the remaining categories in both groups.For Fisher's Exact Test = 2.931 and p>0.05(p=0.798) / Monte Carlo Sig.(2-sided) / 0.788-0.809 / there is no significant difference in the children's responses between the two groups.

Table 11. Frequency of milk with sugar consumption by group

			Group		Total
			Control	ASD	
Q10.7	Never	Count	35	35	70
		%	77,8%	77,8%	77,8%
	Several times a month	Count	2	2	4
		%	4,4%	4,4%	4,4%
	Once a week	Count	3	1	4
		%	6,7%	2,2%	4,4%
	Several times a week	Count	2	5	7
		%	4,4%	11,1%	7,8%
	Every day	Count	2	1	3
		%	4,4%	2,2%	3,3%
	Several times a day	Count	1	1	2
		%	2,2%	2,2%	2,2%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

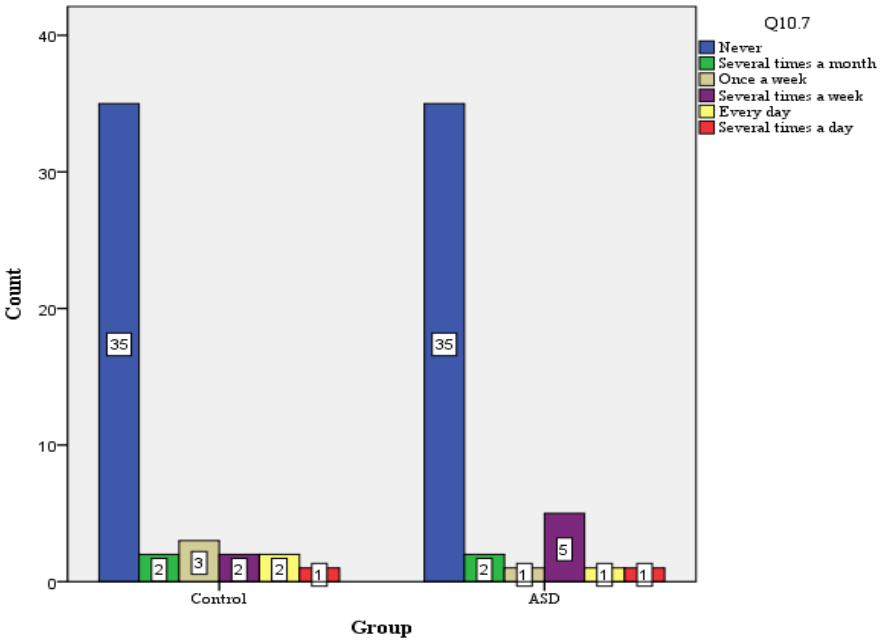

Figure 12. Percentage distribution of milk with sugar consumption by group

The frequency of tea with sugar consumption is presented in Table 12 and Figure 13.

In both groups, most children reported that they never consumed tea with sugar. This response was reported by 29 children, 64.4%, in the control group and 28 children, 62.2%, in the ASD group. The remaining responses were distributed across lower frequency categories in both groups.For Fisher's Exact Test = 2.855 and p>0.05(p=0.805) / Monte Carlo Sig.(2-sided) / 0.795-0.815 / there is no significant difference in the children's responses between the two groups.

Table 12. Frequency of tea with sugar consumption by group

			Group		Total
			Control	ASD	
Q10.8	Never	Count	29	28	57
		%	64,4%	62,2%	63,3%
	Several times a month	Count	5	7	12
		%	11,1%	15,6%	13,3%
	Once a week	Count	3	2	5
		%	6,7%	4,4%	5,6%
	Several times a week	Count	3	3	6
		%	6,7%	6,7%	6,7%
	Every day	Count	5	3	8
		%	11,1%	6,7%	8,9%
	Several times a day	Count	0	2	2
		%	0,0%	4,4%	2,2%
	Total	Count	45	45	90
		%	100,0%	100,0%	100,0%

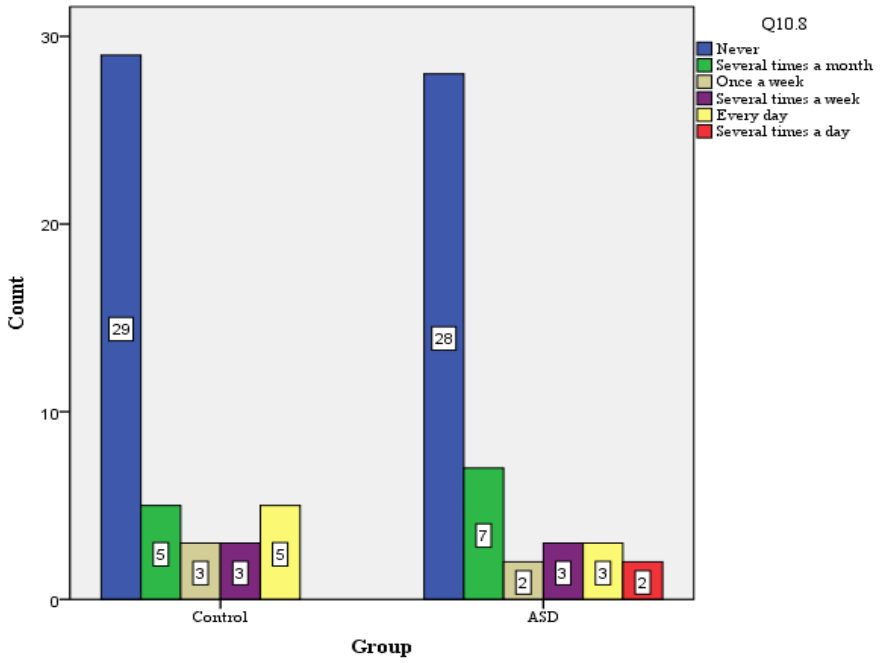

Figure 13. Percentage distribution of tea with sugar consumption by group

The frequency of coffee with sugar consumption is presented in Table 13. Almost all children in both groups reported that they never consumed coffee with sugar. In the control group, all 45 children, 100.0%, answered never, while in the ASD group, 44 children, 97.8%, answered never and only 1 child, 2.2%, reported consuming coffee with sugar several times a month. For Fisher's Exact Test / Exact Sig. (2-sided) p>0.05 (p=1.00), there is no significant difference between the responses of the children in the two groups.

Table 13. Frequency of coffee with sugar consumption by group

			Coffee with sugar (Q10.9)		Total
			Never	Several times a month	
Group	Control	Count	45	0	45
		%	100,0%	0,0%	100,0%
	ASD	Count	44	1	45
		%	97,8%	2,2%	100,0%
Total		Count	89	1	90
		%	98,9%	1,1%	100,0%

Overall, most dietary habits did not differ significantly between the control and ASD groups. A statistically significant difference was observed only for chewing gum with sugar consumption, which was less frequent among children with ASD compared with children in the control group.

4.1.4. Sensory-related oral hygiene difficulties

After the analysis of dietary habits, sensory-related oral hygiene difficulties were evaluated as potential factors influencing oral hygiene maintenance and access to dental care. These factors are particularly relevant for children with ASD, as sensory sensitivity and behavioral difficulties may affect tooth brushing and dental visits. The need for assistance during tooth brushing is presented in Table 14.

The need for assistance during tooth brushing is presented in Table 14. In the control group, 25 children, 55.6%, brushed their teeth independently, while 16 children, 35.6%, sometimes needed help and 4 children, 8.9%, always needed help. In contrast, only 1 child, 2.2%, in the ASD group brushed independently, while 15 children, 33.3%, sometimes needed help and 29 children, 64.4%, always needed help.For Pearson Chi-Square = 41.125 and p<0.001(p=0.000) / Monte Carlo Sig.(2-sided) / 0.000-0.000 / there is a significant difference in the responses of the children between the two groups.

Table 14. Need for assistance during tooth brushing by group

			Does your child need help brushing their teeth? (Q15)			Total
			No, brushes independently	Yes, sometimes	Yes, always	
Group	Control	Count	25	16	4	45
		%	55,6%	35,6%	8,9%	100,0%
	ASD	Count	1	15	29	45
		%	2,2%	33,3%	64,4%	100,0%
Total		Count	26	31	33	90
		%	28,9%	34,4%	36,7%	100,0%

The presence of difficulties during tooth brushing is presented in Table 15. In the control group, 40 children, 88.9%, had no difficulty brushing their teeth, while 5 children, 11.1%, had difficulties. In the ASD group, 38 children, 84.4%, had difficulty brushing their teeth, while only 7 children, 15.6%, reported no difficulty.

Pearson's Chi-Square test showed a statistically significant difference between the groups regarding tooth brushing difficulties, $\chi^2 = 48.496$, $p < 0.001$. These results indicate that difficulties during tooth brushing were significantly more frequent among children with ASD compared with children in the control group.

Table 15. Difficulty in toothbrushing among children in the ASD and control groups

			Does your child have difficulty brushing their teeth? (Q16)		Total
			No	Yes	
Group	Control	Count	40	5	45
		%	88,9%	11,1%	100,0%
	ASD	Count	7	38	45
		%	15,6%	84,4%	100,0%
Total		Count	47	43	90
		%	52,2%	47,8%	100,0%

The presence of difficulties during dental visits due to behavior or sensory sensitivity is presented in Table 16, In the control group, 32 children, 71.1%, had no difficulty visiting the dentist, while 13 children, 28.9%, had difficulties. In the ASD group, 39 children, 86.7%, had difficulty visiting the dentist due to behavior or sensory sensitivity, while only 6 children, 13.3%, reported no difficulty.

Pearson's Chi-Square test showed a statistically significant difference between the groups regarding difficulties during dental visits, $\chi^2 = 30.789$, $p < 0.001$. These results indicate that children with ASD experienced significantly more difficulties during dental visits compared with children in the control group.

Table 16. Difficulties during dental visits due to behavior or sensory sensitivity by group

			Has your child had difficulty visiting the dentist due to behavior or sensory sensitivity? (Q17)		Total
			No	Yes	
Group	Control	Count	32	13	45
		%	71,1%	28,9%	100,0%
	ASD	Count	6	39	45
		%	13,3%	86,7%	100,0%
Total		Count	38	52	90
		%	42,2%	57,8%	100,0%

4.2. Clinical Dental Examination

The clinical examinations performed in this study assessed dental caries, the dmft Index, the GI, the PI, and the BEWE. The findings revealed no significant differences in any of these measures between the study groups. While the mean GI, PI, and BEWE values were slightly higher in the ASD group than in the control group, the differences were not statistically significant (Table 17).

In addition to the simple group comparisons, analyses of correlations between the dental indices were also conducted. In control group, there was a tendency for a positive correlation ($r = 0.29$, $p = 0.057$) between the dmft index and the GI, suggesting that higher dental caries levels were linked to more severe gingival conditions. On the other hand in the ASD group, a strong positive correlation ($r=0.54$, $p<0.001$) was observed between the dmft index and the Gingival Index (GI), indicating that higher levels of dental caries are associated with more severe gingival conditions. Moreover, a weak association was found between enamel wear and plaque accumulation both in the control group, where the correlation coefficient was $r = –0.09$ with a p-value of 0.571, and in the ASD group, where the correlation coefficient was $r = –0.13$ with a p-value of 0.387.

Table 17. Comparison of oral health indices between the control and ASD groups

Variable	N	Control			ASD			P-value
		Mean (SD)	(95%CI)	Median (Min-Max)	Mean (SD)	(95%CI)	Median (Min-Max)	
dmft index	45	5.11(4.42)	(3.78-6.44)	4.00(0.00-14.00)	5.38(3.81)	(4.23-6.52)	4.00(0.00-14.00)	0.54
GI	45	1.44(0.59)	(1.27-1.62)	1.00(0.00-3.00)	1.62(0.72)	(1.41-1.84)	1.00(1.00-3.00)	0.34
PI	45	1.44(0.59)	(1.27-1.62)	1.00(0.00-2.00)	1.56(0.66)	(1.36-1.75)	1.00(1.00-3.00)	0.68
BEWE index	45	3.93(2.03)	(3.32-4.54)	4.00(0.00-9.00)	4.98(3.85)	(3.82-6.14)	4.00(2.00-17.00)	0.8

ASD - autism spectrum disorder; dmf - decay missed filled; GI - gingival index; PI - plaque Index; Bewe - basic erosive wear index

4.3. *Candida albicans*

Table 18 shows the results regarding the presence of *Candida albicans* in the oral cavity of children in both groups.

Of the 45 children in the control group, 40 (88.9%) did not have *Candida albicans* in the oral cavity, and 5 (11.1%) did have *Candida albicans* in the oral cavity.

Of the 45 children in the ASD group, 29 (64.4%) did not have *Candida albicans* in the oral cavity and 16 (35.6%) did have *Candida albicans* in the oral cavity.

Pearson Chi-Square / Asymp. Sig.(2-sided) = 7.516 and $p<0.01$($p=0.006$), there is a significant difference regarding the presence of *Candida albicans* in the oral cavity between the two groups of children.

Table 18. Presence of *Candida albicans* in both group

			Candida albicans		Total
			Candida negative	Candida positive	
Group	Control	Count	40	5	45
		%	88.90%	11.10%	100,0%
	ASD	Count	29	16	45
		%	64.40%	35.60%	100,0%
Total		Count	69	21	90
		%	76,7%	23.30%	100.00%

ASD - autism spectrum disorders

4.3.1. *Candida albicans* (CFU/mL) / Control group

Figure 14 shows the descriptive statistics of the number of *C. albicans* colonies from the buccal mucosa of the children in the control group.

The number of *C. albicans* colonies varied in the interval 55.56±15.89 CFU/mL; ±95.00%CI:50.78-60.33; the median was 50.00 CFU/mL; the minimum value was 50.00 CFU/mL, and the maximum value was 100.00 CFU/mL.

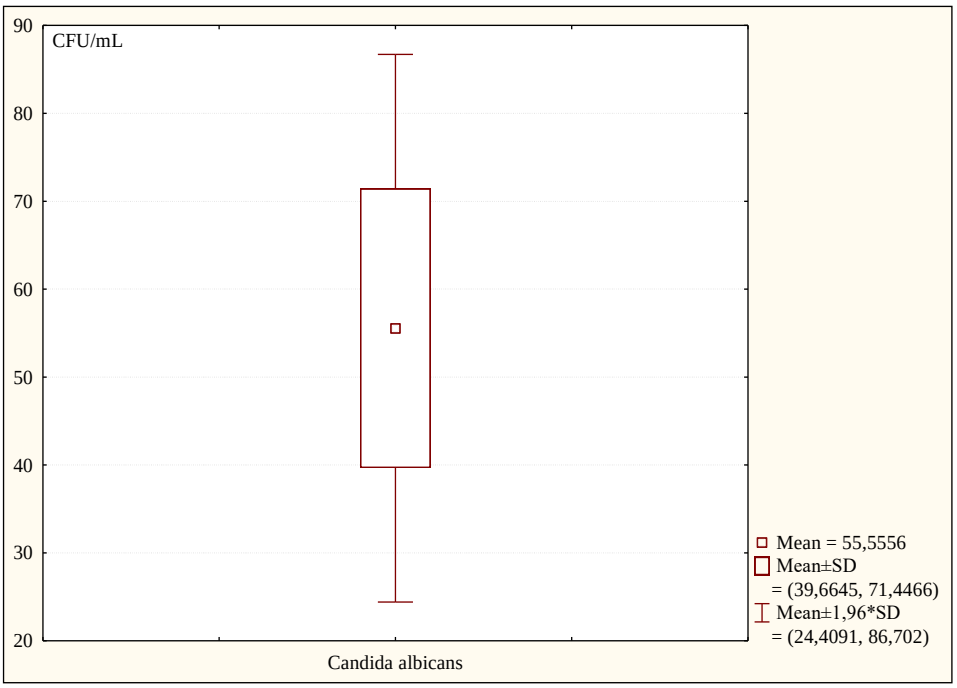

Figure 14. *Candida albicans* colony count (CFU/mL) in the control group

4.3.2. *Candida albicans* (CFU/mL) / ASD group

Figure 15 shows the descriptive statistics of the number of *C. albicans* colonies from the buccal mucosa of children in the ASD group.

The number of *C. albicans* colonies varied in the interval 67.78±24.20 CFU/mL; ±95.00%CI:60.51-75.05; the median was 50.00 CFU/mL; the minimum value was 50.00 CFU/mL, and the maximum value was 100.00 CFU/mL.

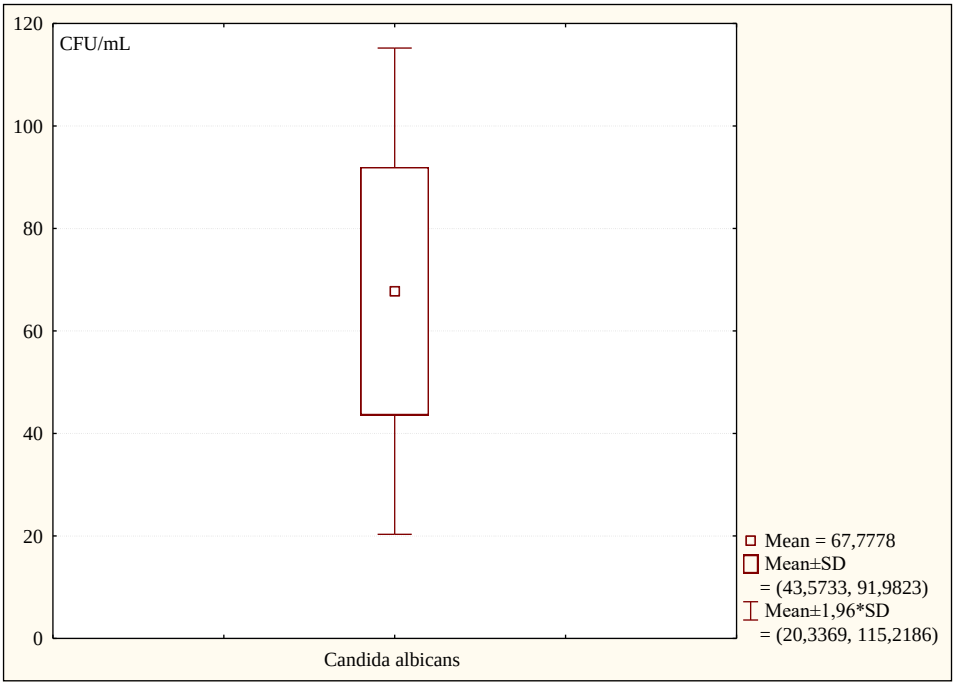

Figure 15. *Candida albicans* colony count (CFU/mL) in the ASD group

4.3.3. Differences / Control group and ASD group

The number of *Candida albicans* colonies from the buccal mucosa of the children in the ASD group was significantly higher than the number of Candida albicans colonies from the buccal mucosa of the children in the control group where $Z= 2.73$ and $p<0.01$ ($p=0.006$).

4.4. Hormonal Status

Table 19 shows the comparison of cortisol and melatonin levels between the control and ASD groups. The ASD group had significantly higher cortisol levels than the control group (3.60 ± 4.54 vs. 1.13 ± 1.07 ng/mL; $p < 0.001$). Melatonin levels were significantly higher in the control group than in the ASD group (15.27 ± 10.10 vs. 11.16 ± 8.76 pg/mL; $p = 0.04$). Data are presented as mean ± SD, 95% CI, and median (minimum maximum). Group differences were analyzed using the Mann Whitney U test.

Table 19. Cortisol and melatonin levels in the control and ASD groups

Variable	N	Control			ASD			Rank Sum Control	Rank Sum ASD	U	Z adjusted	P value
		Mean (SD)	(95% CI)	Median (Min Max)	Mean (SD)	(95% CI)	Median (Min Max)					
Cortisol	5	1.13 (1.07)	(0.81 to 1.45)	0.85 (0.04 to 6.20)	3.60 (4.54)	(2.23 to 4.96)	2.10 (0.15 to 27.40)	1402	2693	367	5.21	<0.001
Melatonin	45	15.27 (10.10)	(12.23 to 18.30)	14.90 (0.57 to 40.20)	11.16 (8.76)	(8.53 to 13.79)	10.60 (0.01 to 35.20)	2295	1800	765	-1.99	0.04

ASD - autism spectrum disorder; SD - standard deviation; CI - confidence interval; U - Mann Whitney; Z - Standardized Test

4.5. Parent/Guardian Perceptions Questionnaire (P-CPQ-16)

The P-CPQ-16 assesses how parents or caregivers perceive the effect of children's oral health on their quality of life. The questionnaire comprises four subscales. Table 20 presents the mean values of each subscale for each group. The mean oral symptoms were higher in the ASD group but the difference was not statistically significant ($p = 0.115$). In contrast, the ASD group reported greater functional limitations than the control group ($p < 0.001$). Similarly, the level of emotional impairment was higher in the ASD group than in the control group ($p < 0.001$), as was social impairment ($p < 0.001$). Overall, the mean P-CPQ-16 score was significantly higher in the ASD group than in the control group ($p < 0.001$) (Table 4). The findings for the P-CPQ-16 subscales revealed that parents/caregivers of children with ASD noted that their children had greater functional limitations, more intense emotional distress and social stress implicating their

quality of life than the children in control group. The differences were significant for all subscales except for oral symptoms (Table 20).

Table 20. Comparison of Parent/Guardian Perceptions Questionnaire netween Control and ASD Groups

			Control			ASD			
	Variable	N	Mean (SD)	(95%CI)	Median (Min-Max)	Mean (SD)	(95%CI)	Median (Min-Max)	P-value
Oral symptoms	Total	45	3.60(2.41)	(2.88-4.32)	3.00(0.00-9.00)	4.33(2.13)	(3.69-4.97)	4.00(0.00-8.00)	0.15
	Mean	45	1.20(0.80)	(0.96-1.44)	1.00(0.00-3.00)	1.44(0.71)	(1.23-1.66)	1.33(0.00-2.67)	
Functional limitations	Total	45	3.16(2.70)	(2.35-3.97)	3.00(0.00-9.00)	9.20(2.95)	(8.31-10.09)	10.00(2.00-15.00)	**0.000**
	Mean	45	1.05(0.90)	(0.78- 1.32)	1.00(0.00-3.00)	2.30(0.74)	(2.08-2.52)	2.50(0.50-3.75)	
Emotional well-being	Total	45	2.60(1.42)	(2.17- 3.03)	3.00(0.00-4.00)	9.04(1.77)	(8.51-9.58)	9.00(4.00-13.00)	**0.000**
	Mean	45	1.30(0.71)	(1.09- 1.51)	1.50(0.00-2.00)	2.26(0.44)	(2.13-2.39)	2.25(1.00-3.25)	
Social well-being	Total	45	0.29(0.82)	(0.04- 0.53)	0.00(0.00-4.00)	9.76(1.46)	(9.32-10.20)	10.00(6.00-12.00)	**0.000**
	Mean	45	0.14(0.41)	(0.02- 0.27)	0.00(0.00-2.00)	3.25(0.49)	(3.11-3.40)	3.33(2.00-4.00)	
P-CPQ-16	Total	45	9.64(5.27)	(8.06-11.23)	9.00(0.00-25.00)	32.33 (5.89)	(30.56-34.10)	33.00(17.00-41.00)	**0.000**
	Mean	45	0.96(0.53)	(0.81-1.12)	0.90(0.00 2.50)	2.31(0.42)	(2.18-2.44)	2.36(1.21-2.93)	

ASD - autism spectrum disorder; SD - standard deviation; P-CPQ-16 - Parent/Guardian Perceptions Questionnaire, 16-item version.

4.6. Relationship between hormone levels, *Candida albicans*, and oral health parameters

4.6.1. Control Group

4.6.1.1. Single correlation analysis

In relation to the main objective of examining the association between hormone levels and oral health, correlation analyses were performed in the control group. The correlations included cortisol, melatonin, *Candida albicans* colony count, and oral health parameters such as dmft index, Gingival Index, Plaque Index, and BEWE Index.

Figure 16 shows the correlation between the dmft index and *Candida albicans* (CFU/mL). An increase in the number of *Candida albicans* colonies was accompanied by an increase in the dmft index. With R = 0.17 and p>0.05 (p=0.272), a weak non-significant correlation was determined.

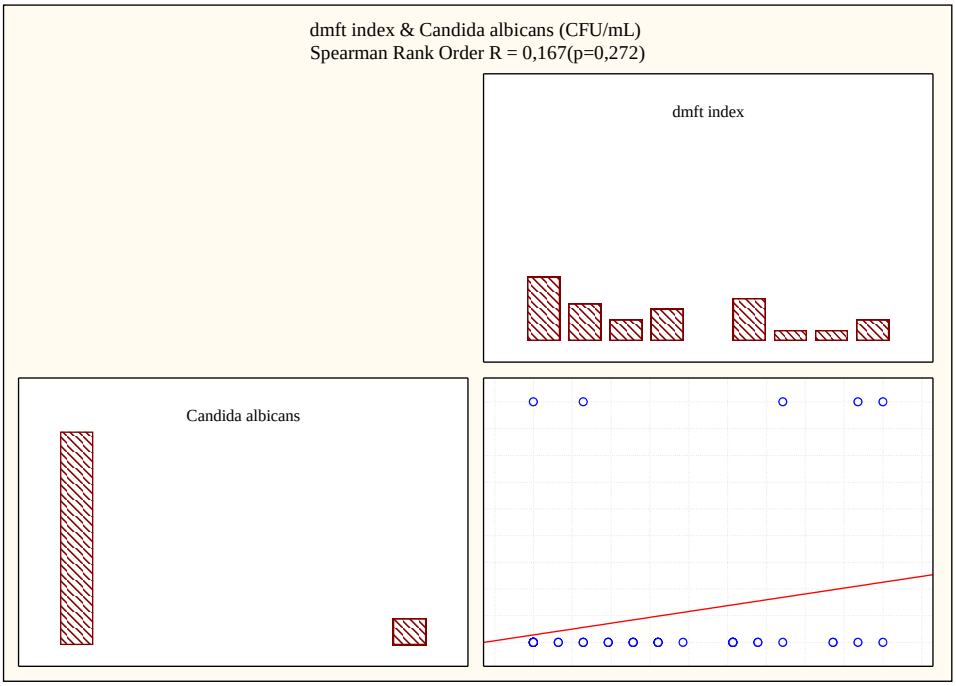

Figure 16. Correlation between dmft index and *Candida albicans* colony count in the control group

Figure 17 shows the correlation between the Gingival Index (GI) and *Candida albicans* (CFU/mL). An increase in the number of Candida albicans colonies was accompanied by an increase in the Gingival Index (GI). With R = 0.24 and p>0.05 (p=0.115), a weak non-significant correlation was determined.

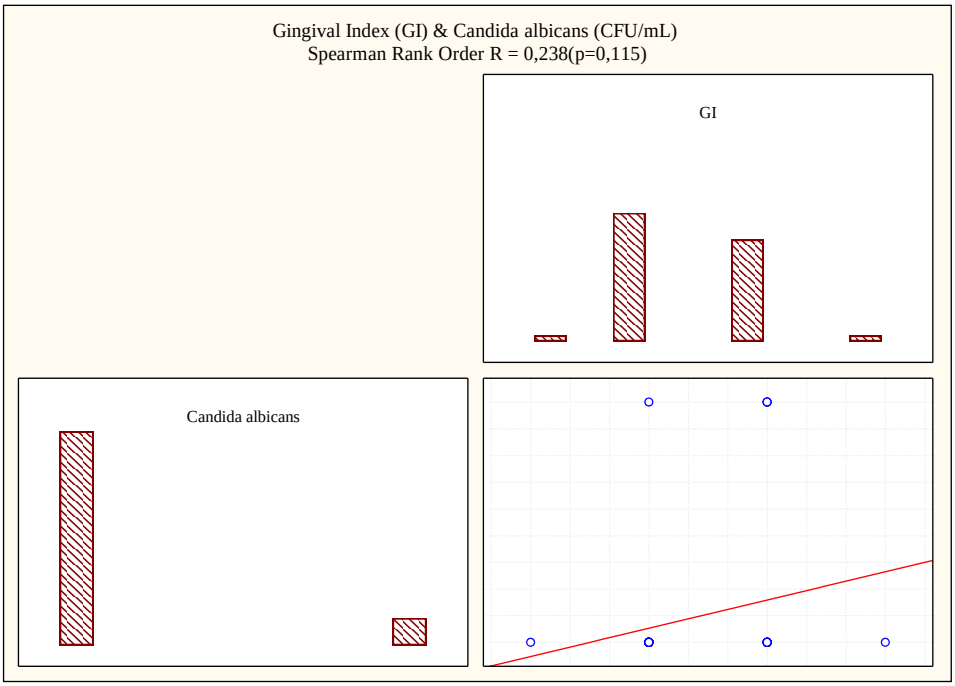

Figure 17. Correlation between GI index and *Candida albicans* colony count in the control group

Figure 18 shows the correlation between the Plaque Index (PI) and *Candida albicans* (CFU/mL). The increase in the number of *Candida albicans* colonies was accompanied by an increase in the Plaque Index (PI). With R = 0.35 and p<0.05 (p=0.017), a moderately strong, significant correlation was determined.

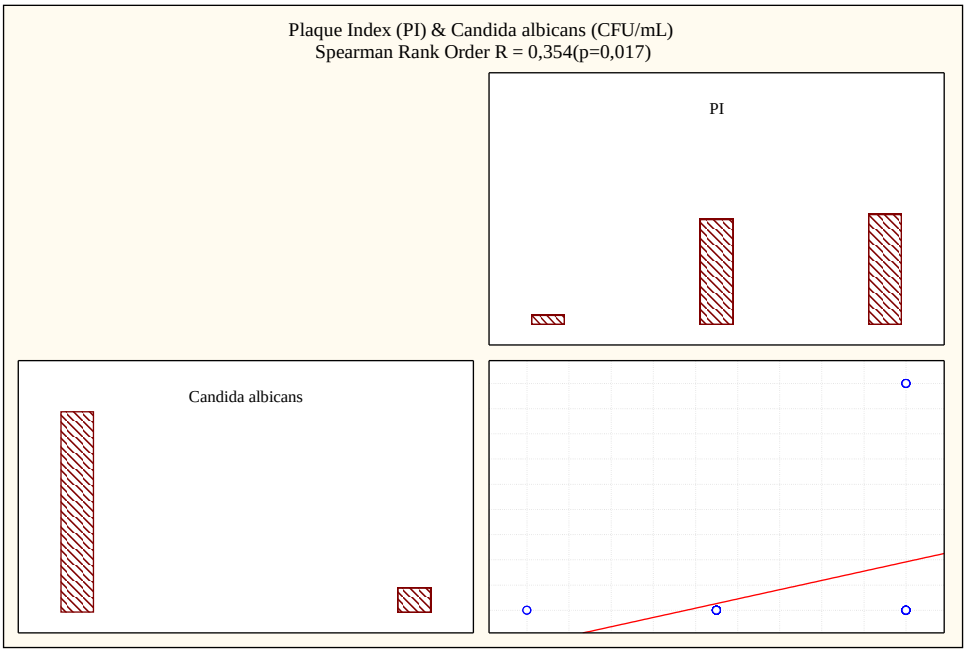

Figure 18. Correlation between Plaque Index and *Candida albicans* colony count in the control group

Figure 19 shows the correlation between the BEWE index and *Candida albicans* (CFU/mL). An increase in the number of *Candida albicans* colonies was accompanied by a decrease in the BEWE index. With R = -0.18 and p>0.05 (p=0.241), a moderately weak, negative, insignificant correlation was determined.

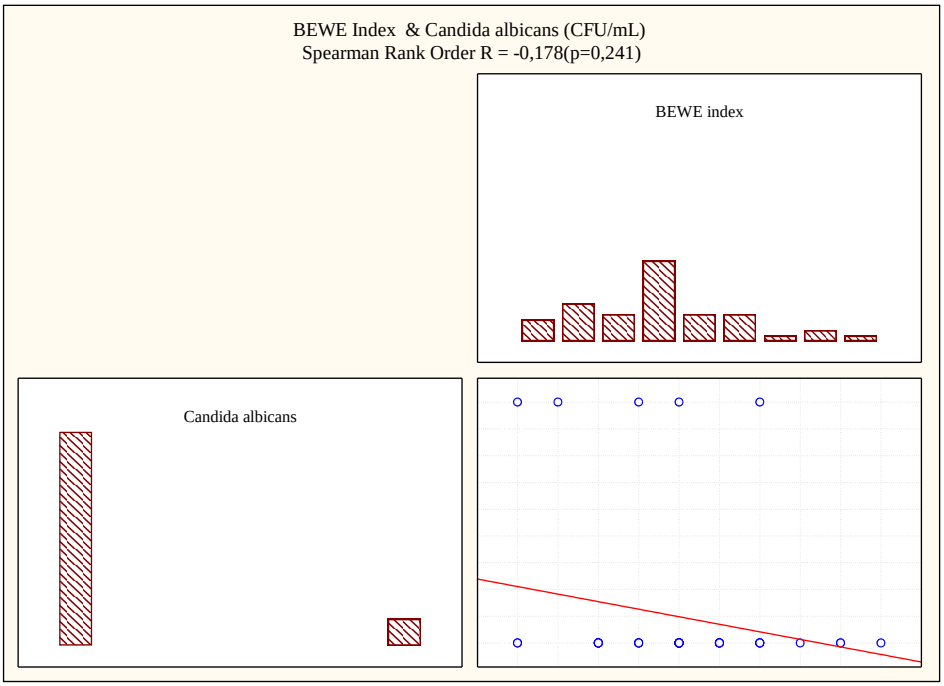

Figure 19. Correlation between BEWE Index and Candida albicans colony count in the control group

Figure 20 shows the correlation between the dmft index and Cortisol ng/mL. The increase in the value of Cortisol ng/mL is accompanied by a decrease in the value of dmft index. For R = -0.40 and p<0.01 (p=0.007) a moderately strong, negative, significant correlation was determined.

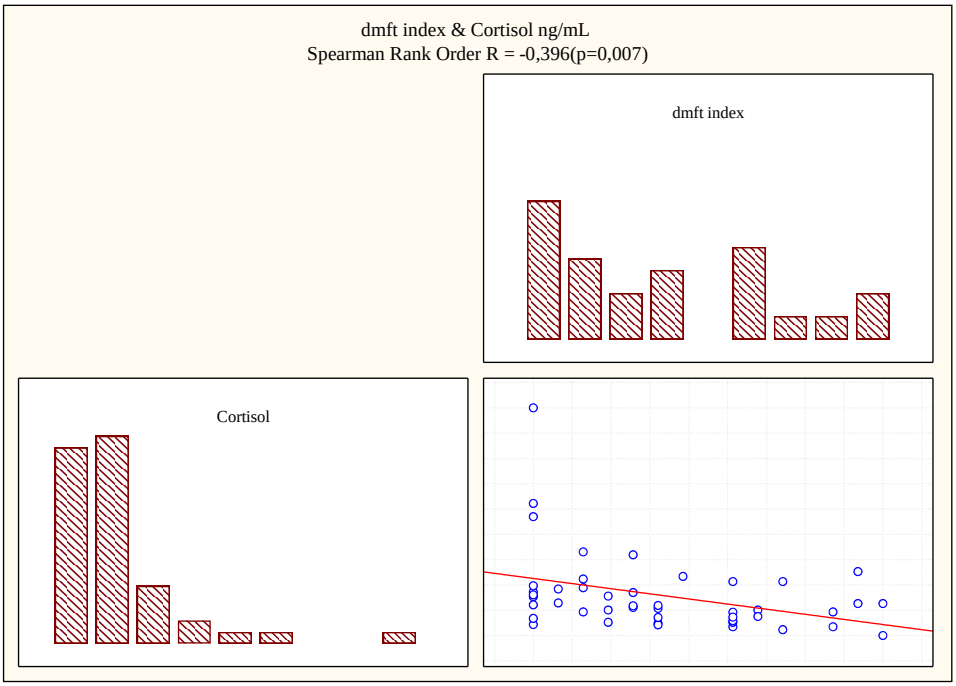

Figure 20. Correlation between dmft index and cortisol levels in the control group

Figure 21 shows the correlation between the Gingival Index (GI) and cortisol ng/mL. An increase in the value of cortisol ng/mL was accompanied by a decrease in the Gingival Index (GI). With R = -0.24 and p>0.05 (p=0.114), a weak negative, insignificant correlation was determined.

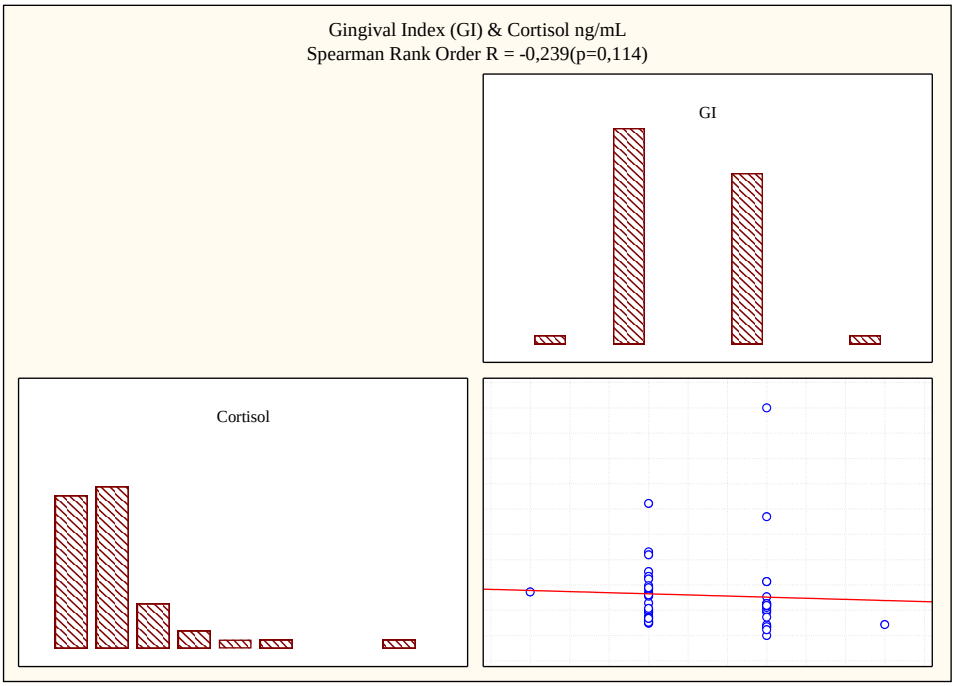

Figure 21. Correlation between Gingival Index and cortisol levels in the control group

Figure 22 shows the correlation between the Plaque Index (PI) and cortisol ng/mL. An increase in the value of cortisol ng/mL was accompanied by a decrease in the Plaque Index (PI). With R = -0.12 and p>0.05 (p=0.445), a weak, negative, insignificant correlation was determined.

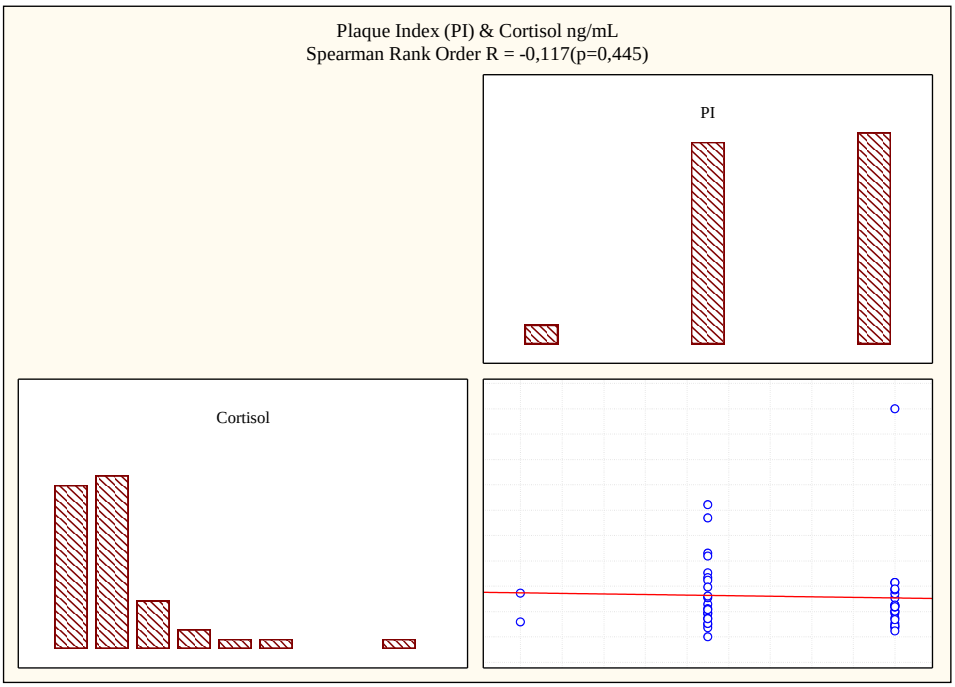

Figure 22. Correlation between Plaque Index and cortisol levels in the control group

Figure 23 shows the correlation between the BEWE Index and cortisol ng/mL. An increase in the value of cortisol ng/mL was accompanied by a decrease in the BEWE Index. With R = -0.19 and p>0.05 (p=0.202), a weak negative, insignificant correlation was determined.

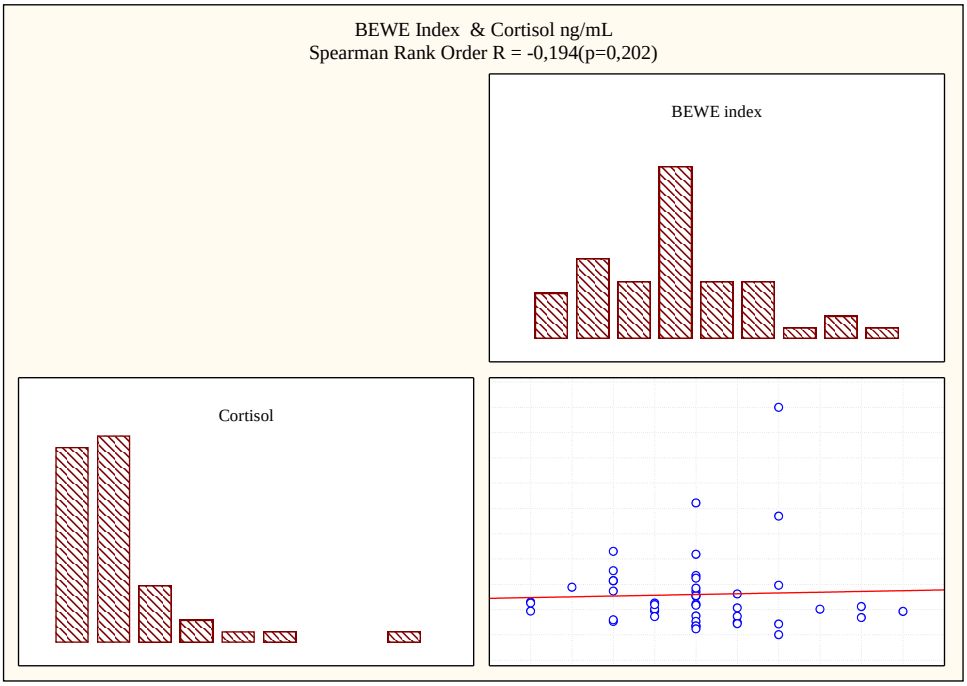

Figure 23. Correlation between BEWE Index and cortisol levels in the control group

Figure 24 shows the correlation between the dmft index and melatonin pg/mL. An increase in the value of melatonin pg/mL was accompanied by a decrease in the dmft index. With R = -0.15 and p>0.05 (p=0.324), a weak negative, insignificant correlation was determined.

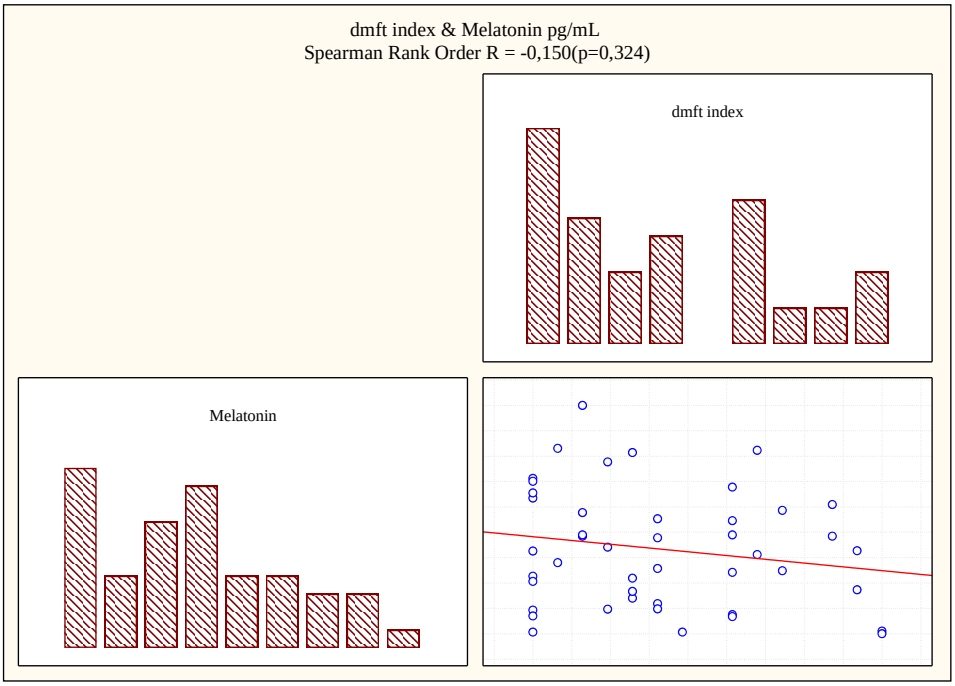

Figure 24. Correlation between dmft index and melatonin levels in the control group

Figure 25 shows the correlation between the Gingival Index (GI) and melatonin pg/mL. An increase in the value of melatonin pg/mL was accompanied by a decrease in the Gingival Index (GI). With R = -0.07 and p>0.05 (p=0.661), a very weak negative, insignificant correlation was determined.

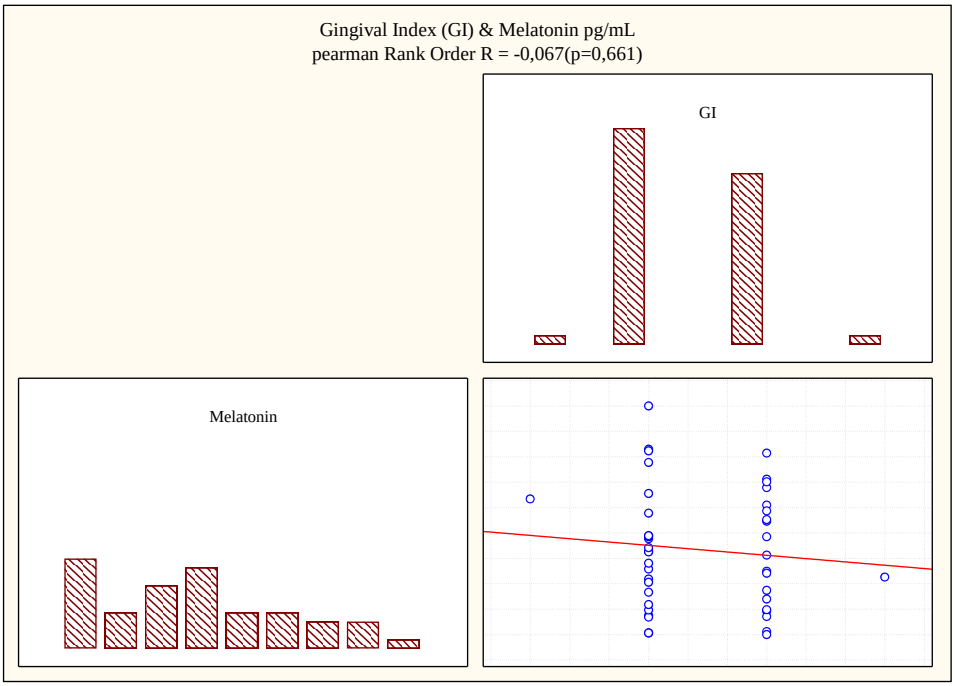

Figure 25. Correlation between Gingival Index and melatonin levels in the control group

Figure 26 shows the correlation between the Plaque Index (PI) and melatonin pg/mL. An increase in the value of Melatonin pg/mL was accompanied by a decrease in the Plaque Index (PI). With R = -0.07 and p>0.05 (p=0.663), a very weak negative, insignificant correlation was determined.

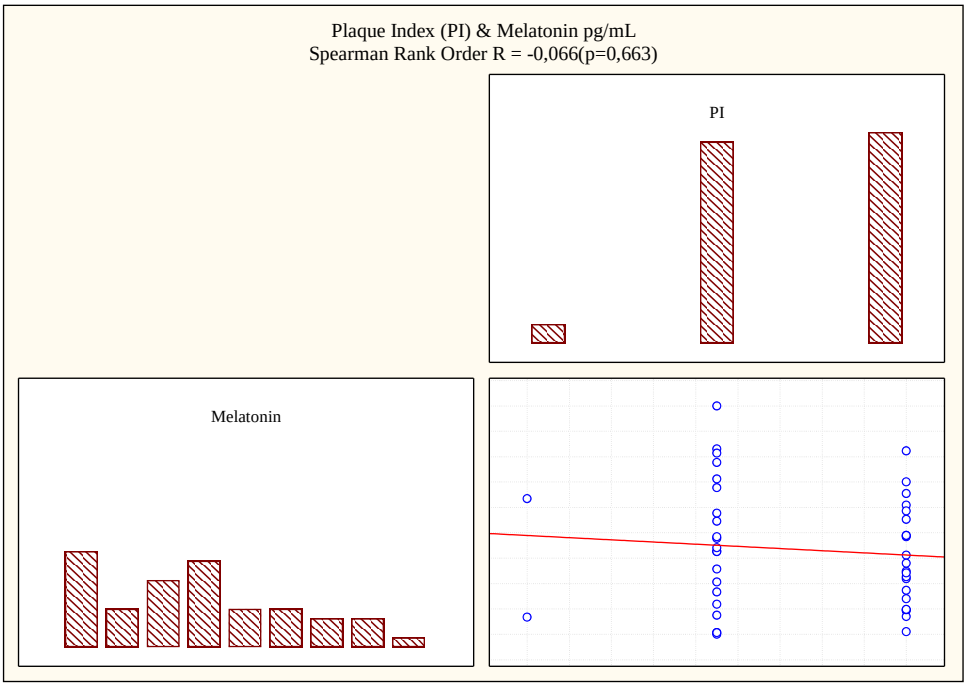

Figure 26. Correlation between Plaque Index and melatonin levels in the control group

Figure 27 shows the correlation between the BEWE Index and melatonin pg/mL. An increase in the value of melatonin pg/mL was accompanied by an increase in the BEWE Index. With R = 0.03 and p>0.05 (p=0.825), a very weak, non-significant correlation was determined.

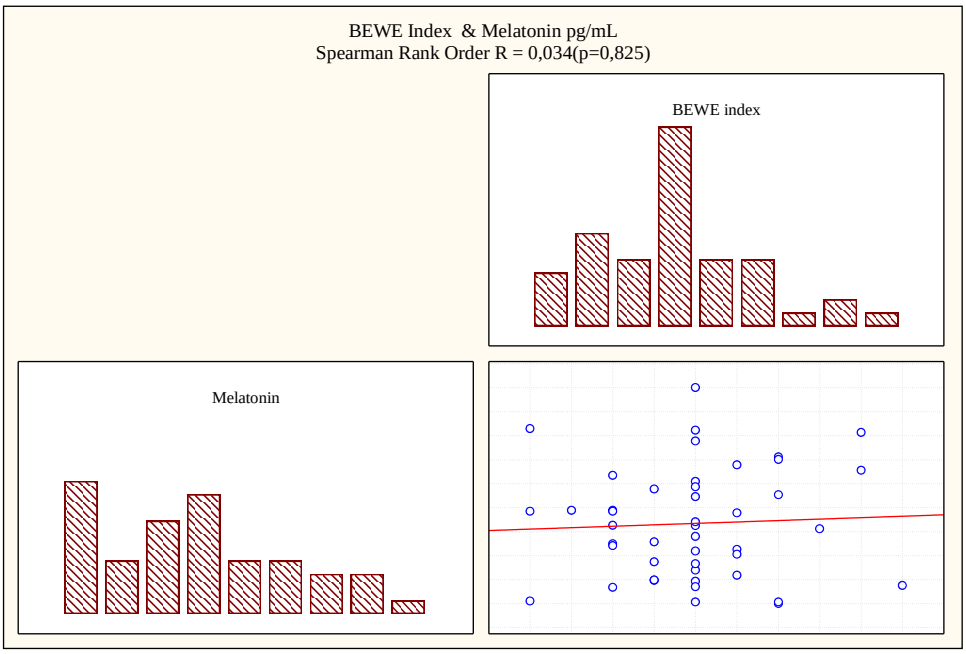

Figure 27. Correlation between BEWE Index and melatonin levels in the control group

Figure 28 shows the correlation between cortisol ng/mL and melatonin pg/mL. An increase in melatonin pg/mL was accompanied by a decrease in cortisol ng/mL. With R = -0.06 and p>0.05 (p=0.679), a very weak negative, insignificant correlation was determined.

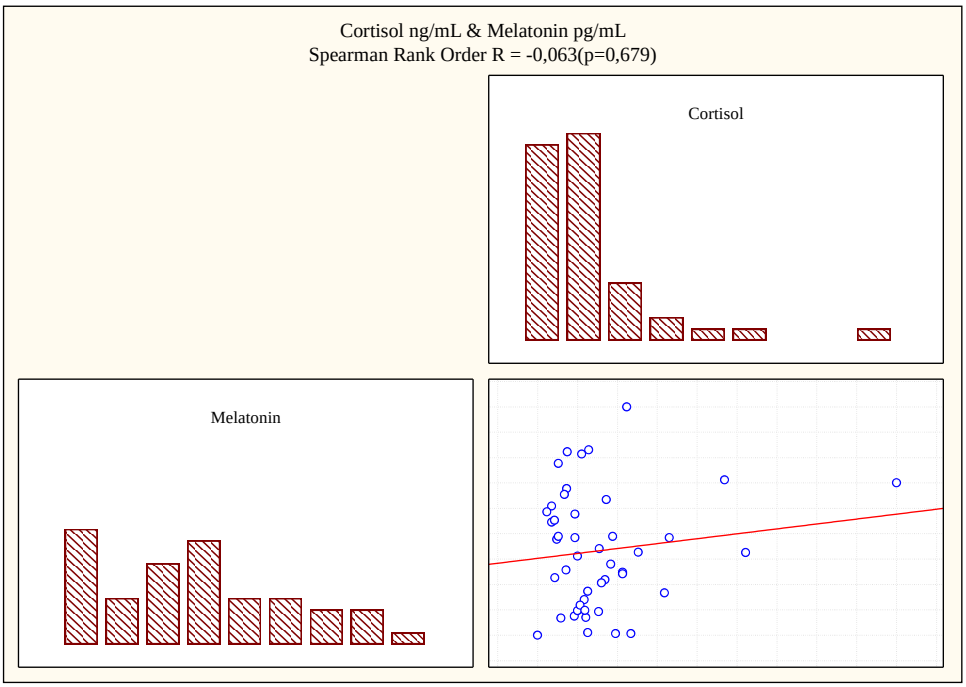

Figure 28. Correlation between cortisol and melatonin levels in the control group

Figure 29 shows the correlation between the Gingival Index (GI) and the dmft index. An increase in the dmft Index was accompanied by an increase in the Gingival Index (GI). With R = 0.29 and p>0.05 (p=0.057), a moderately strong, non-significant correlation was determined.

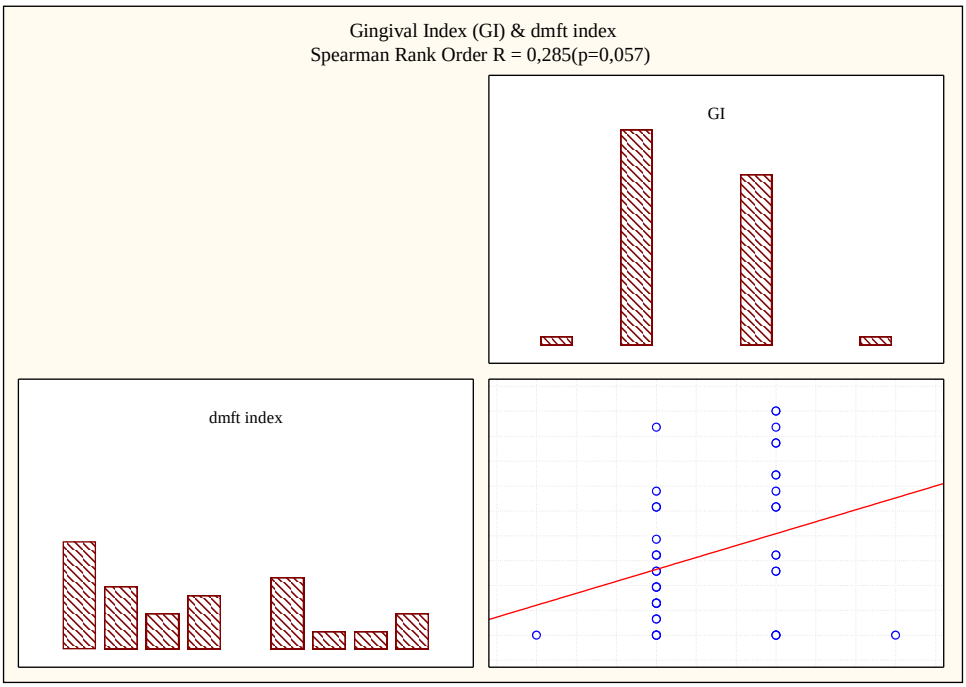

Figure 29. Correlation between Gingival Index and dmft index in the control group

Figure 30 shows the correlation between the BEWE index and the Plaque Index (PI). An increase in the Plaque Index (PI) was accompanied by a decrease in the BEWE index. With R = -0.09 and p>0.05 (p=0.571), a very weak negative, insignificant correlation was determined.

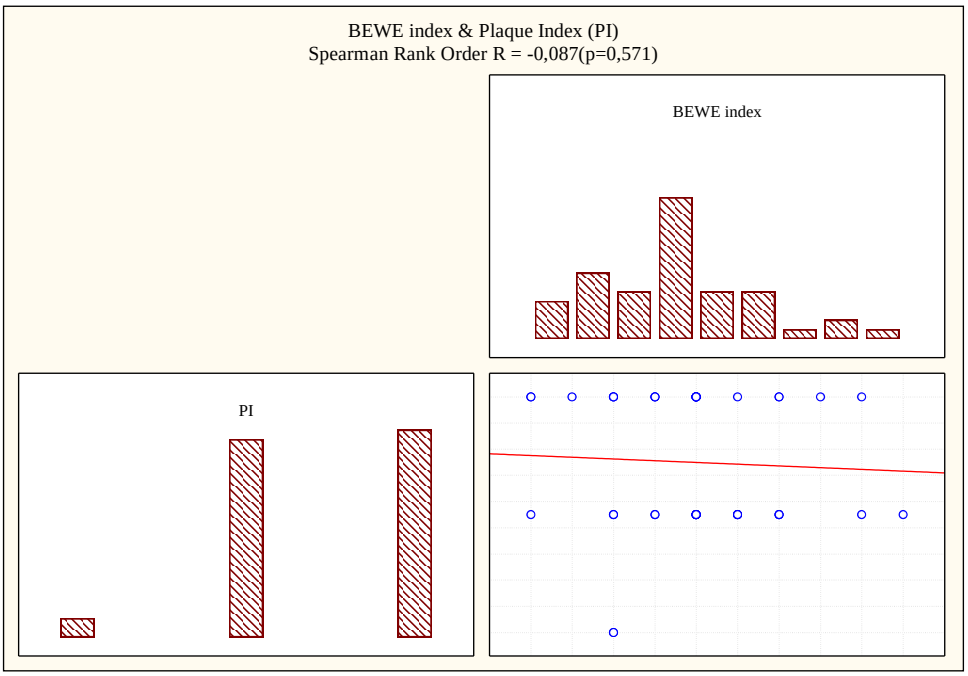

Figure 30. Correlation between BEWE Index and Plaque Index in the control group

Overall, in the control group, most correlations between hormone levels, *Candida albicans* colony count, and oral health parameters were weak and not statistically significant. A statistically significant positive correlation was observed between Plaque Index and *Candida albicans* colony count, indicating that higher plaque accumulation was associated with increased *Candida albicans* colonies. A statistically significant negative correlation was also observed between dmft index and cortisol levels, suggesting that higher cortisol levels were associated with lower dmft index values. No statistically significant correlations were found between melatonin levels and the analyzed oral health parameters.

4.6.1.2. Multiple regression analysis

In relation to the study objective examining the association between hormonal parameters and oral health, multiple linear regression analysis was performed in the control group to identify predictors of the dmft index. The model was statistically significant, R = 0.568, F = 3.706, p = 0.008. Cortisol was the only statistically significant predictor, β = -0.441, p = 0.003, indicating that higher cortisol levels were associated with lower dmft index values. Other variables were not statistically significant predictors (Table 21).

Table 21. Multiple linear regression analysis of predictors of the dmft index in the control group

Model	Unstandardized Coefficients		Standardized	t	Sig.	95,0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	10.093	4.591		2.199	0.034	0.807	19.378
Sex (1)	2.179	1.164	0.249	1.872	0.069	-0.176	4.535
Age	-0.983	0.845	-0.16	-1.163	0.252	-2.693	0.727
Pl	0.377	1.073	0.05	0.351	0.728	-1.794	2.547
CA (1)	4.086	2.087	0.294	1.958	0.057	-0.136	8.308
Cortisol	-1.827	0.577	-0.441	-3.166	0.003	-2.994	-0.66

a. Dependent Variable: dmft index

CA- Candida albicans; PI- plaque index; dmft - decayed, missing, and filled teeth index.

In the second regression model, cortisol was used as the dependent variable, while melatonin, Gingival Index, *Candida albicans* status, and age were included as independent variables. The model was not statistically significant, R = 0.336, F = 1.269, p = 0.298. None of the included variables were statistically significant predictors of cortisol levels in the control group.

In the third regression model, the BEWE Index was used as the dependent variable, while age, Plaque Index, dmft index, and cortisol were included as independent variables. This model was also not statistically significant, R = 0.244, F = 0.631, p = 0.643. None of the included variables were statistically significant predictors of the BEWE Index in the control group.

4.6.1.3. Binary logistic regression for Candida albicans

In relation to the general objective of examining the association between hormonal parameters and oral health, and the specific objective assessing *Candida albicans* prevalence, binary logistic regression was performed to evaluate whether sex, age, dmft index, cortisol, and melatonin predicted the presence of *Candida albicans* in the control group. The model correctly classified 91.1% of cases. Specificity was 100.0%, indicating that all children without *Candida albicans* were correctly identified, while sensitivity was 20.0%, indicating limited ability to identify children with *Candida albicans* positivity (Table 22).

Table 22. Predictors of *Candida albicans* Presence in the Discrimination Model

Observed			Predicted		
			Candida albicans		Percentage Correct
			Candida negative	Candida positive	
Step 1	CA	Candida negative	40	0	100
		Candida positive	4	1	20
		Overall Percentage			91,1
The cut-off value is .500					

ASD - autism spectrum disorder; CA - Candida albicans; dmft - decayed, missing, and filled teeth

None of the included predictors showed a statistically significant independent contribution to the prediction of *Candida albicans* presence in the control group. The strongest predictors were the dmft index, Wald = 3.412, p = 0.065, and cortisol, Wald = 3.204, p = 0.073; however, both remained statistically non-significant. Sex, age, and melatonin were also not statistically significant predictors, Table (22.1).

Table 22.1 Binary Logistic Regression/presence of CA

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1a	Sex (1)	-1.788	1.693	1.115	1	0.291	0.167	0.006	4.622
	Age	-0.743	0.881	0.711	1	0.399	0.476	0.085	2.676
	dmft index	0.373	0.202	3.412	1	0.065	1.452	0.978	2.156
	Cortisol	0.962	0.537	3.204	1	0.073	2.616	0.913	7.495
	Melatonin	0.002	0.062	0.001	1	0.976	1.002	0.887	1.132
	Constant	-1.534	4.795	0.102	1	0.749	0.216		

a. Variable(s) entered at step 1: Sex, Age, dmft, Cortisol, Melatonin.

B - regression coefficient; S.E. - standard error; Sig. - significance level; Exp(B) - odds ratio; 95% C.I. - confidence interval.

The ROC analysis showed an area under the curve of 0.850, 95% CI: 0.641–1.000, p = 0.011. This indicates good overall discriminatory ability of the model for predicting the presence of *Candida albicans* in the control group, Figure (31).

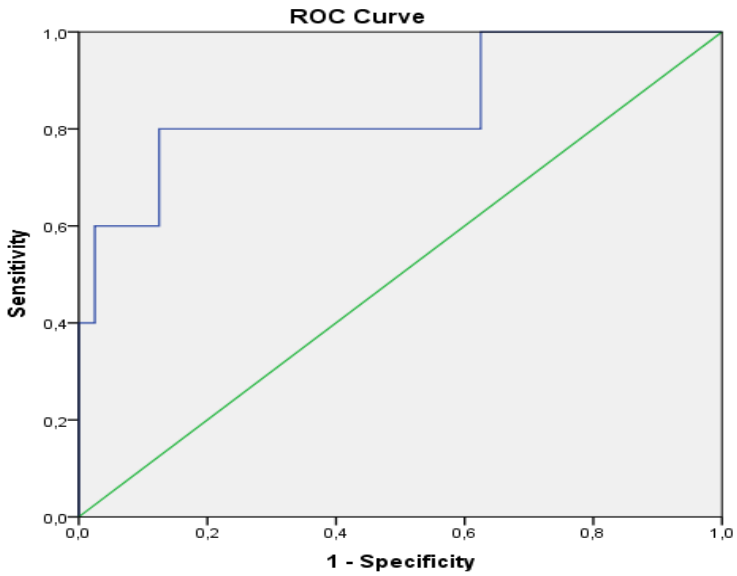

Figure 31. ROC curve for the prediction of *Candida albicans* presence in the control group

Table 22.2. Area under the ROC curve for the prediction of *Candida albicans* presence in the control group

Test Result Variable(s): Predicted probability

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.850	.107	.011	.641	1.000

a - Under the nonparametric assumption; b - Null hypothesis: true area = 0.5.

4.6.2. Autism spectrum disorders group

4.6.2.1. Single correlation analysis

Single correlation analysis was performed to examine the relationships between hormone levels, *Candida albicans* colony count, and oral health parameters in the ASD group.

Figure 32 shows the correlation between the dmft index and *Candida albicans* (CFU/mL). An increase in the number of *Candida albicans* colonies was accompanied by an increase in the dmft index. With R = 0.22 and p>0.05 (p=0.142), a moderately weak non-significant correlation was determined.

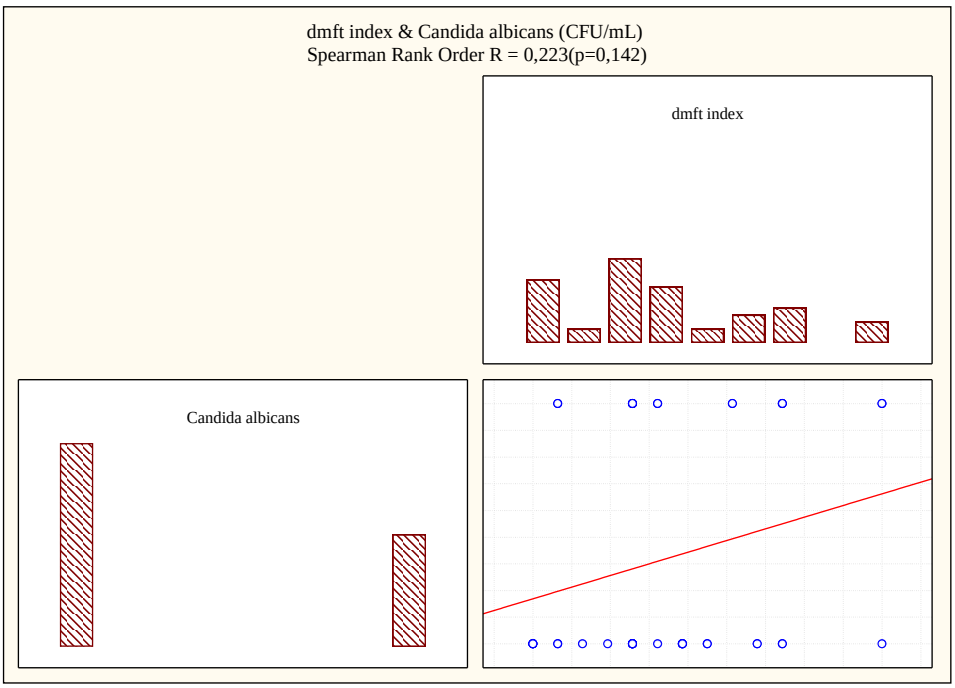

Figure 32. Correlation between dmft index and *Candida albicans* colony count in the ASD group

Figure 33 shows the correlation between the Gingival Index (GI) and *Candida albicans* (CFU/mL). An increase in the number of Candida albicans colonies was accompanied by an increase in the Gingival Index (GI). With R = 0.17 and p>0.05 (p=0.259), a moderately weak, non-significant correlation was determined.

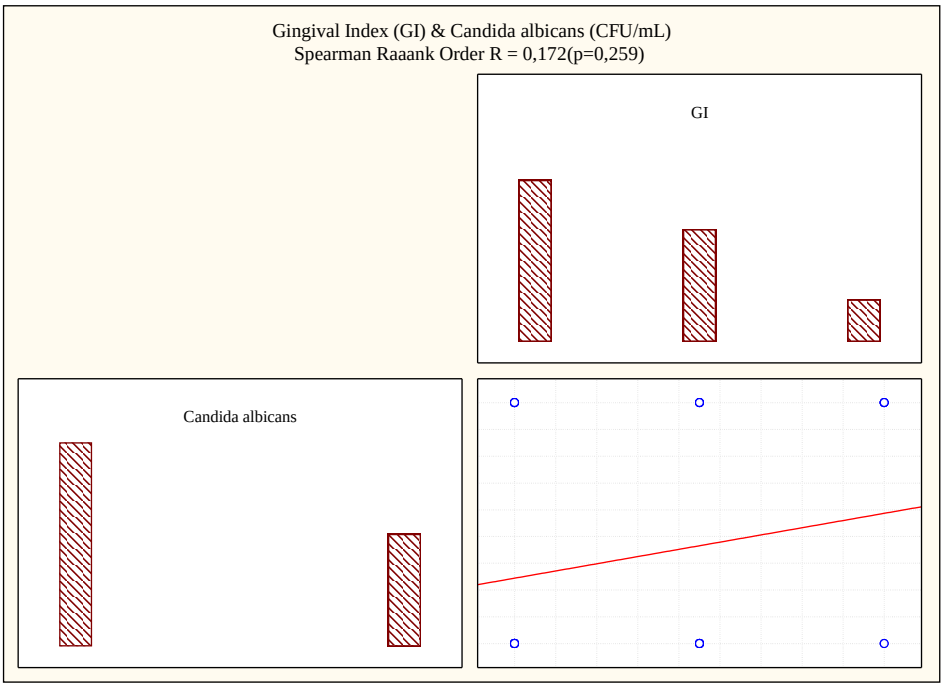

Figure 33. Correlation between Gingival Index and *Candida albicans* colony count in the ASD group

Figure 34 shows the correlation between the Plaque Index (PI) and *Candida albicans* (CFU/mL). An increase in the number of C. albicans colonies was accompanied by an increase in the Plaque Index (PI). With R = 0.07 and p>0.05 (p=0.448), a very weak, non-significant correlation was determined.

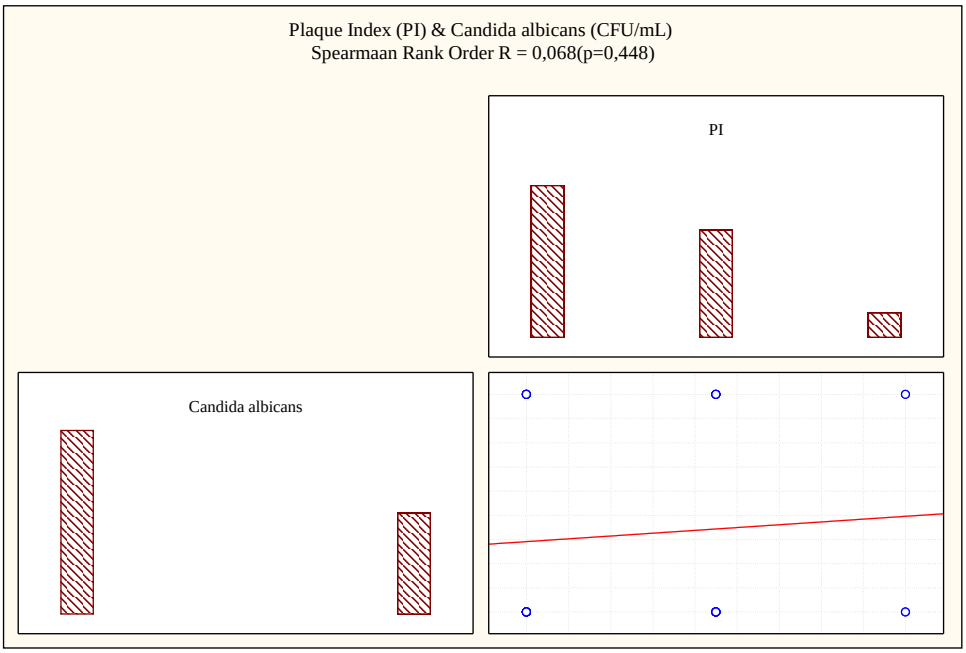

Figure 34. Correlation between Plaque Index and *Candida albicans* colony count in the ASD group

Figure 35 shows the correlation between the BEWE index and *Candida albicans* (CFU/mL). An increase in the number of *Candida albicans* colonies was accompanied by an increase in the BEWE index. With R = 0.16 and p>0.05 (p=0.292), a moderately weak positive, insignificant correlation was determined.

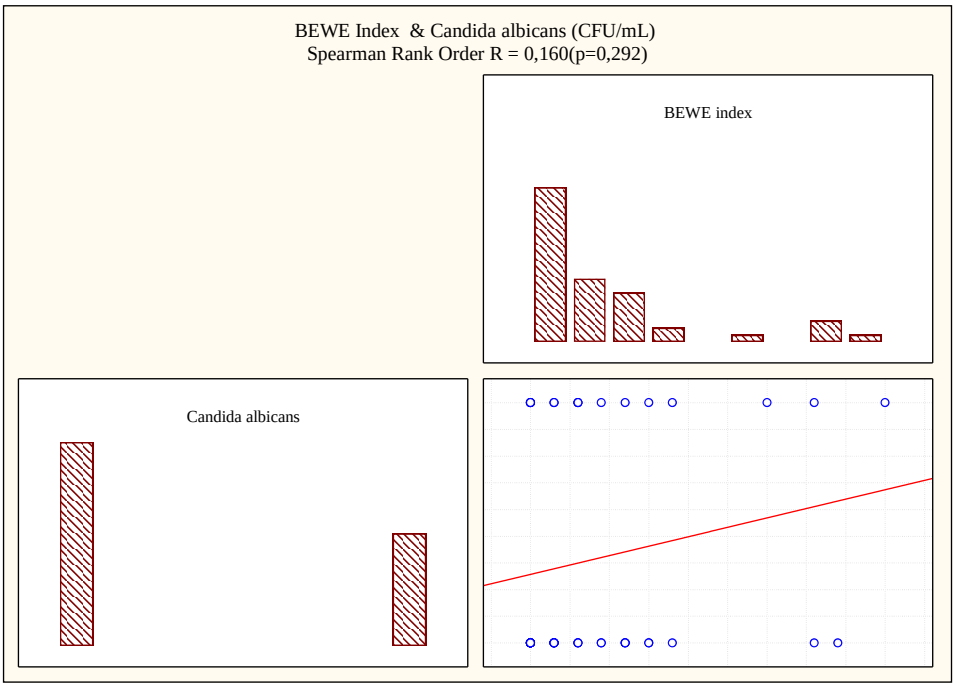

Figure 35. Correlation between BEWE Index and *Candida albicans* colony count in the ASD group

Figure 36 shows the correlation between the dmft index and Cortisol ng/mL. An increase in the amount of Cortisol ng/mL was accompanied by an increase in the dmft index. With R = 0.04 and p>0.05 (p=0.822), a very weak positive, insignificant correlation was determined.

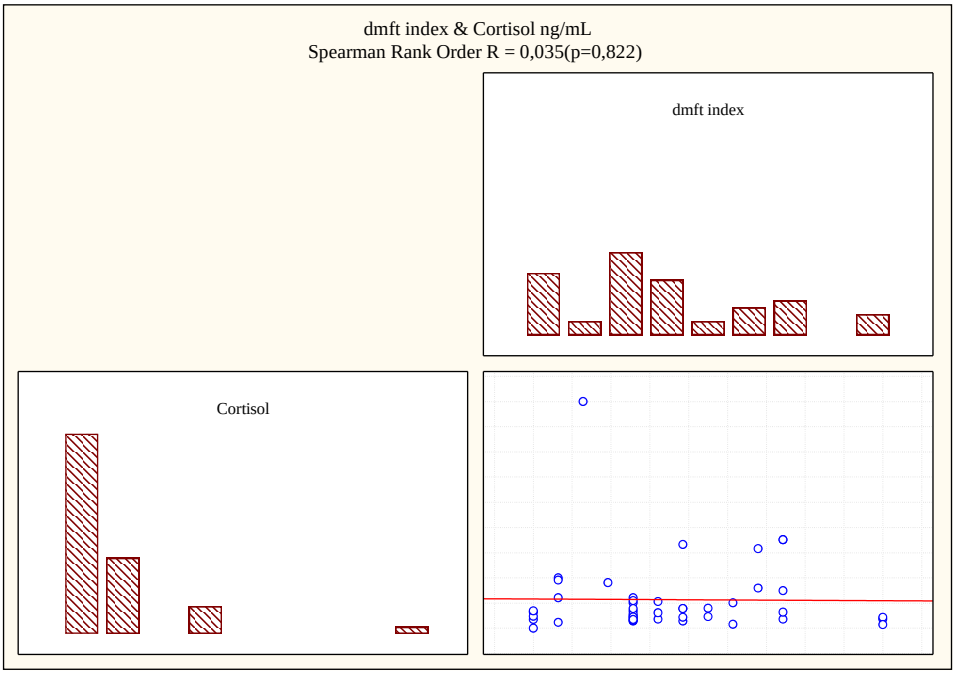

Figure 36. Correlation between dmft index and cortisol levels in the ASD group

Figure 37 shows the correlation between the Gingival Index (GI) and cortisol ng/mL. An increase in cortisol ng/mL was accompanied by an increase in the Gingival Index (GI). With R = 0.05 and p>0.05 (p=0.726), a very weak positive, insignificant correlation was determined.

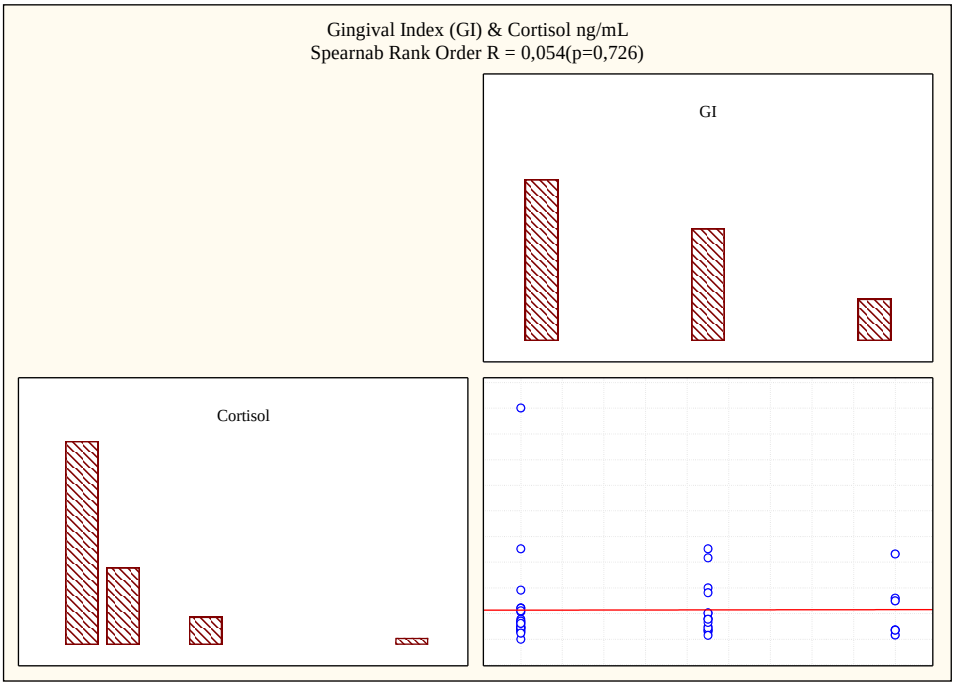

Figure 37. Correlation between GI index and cortisol levels in the ASD group

Figure 38 shows the correlation between the Plaque Index (PI) and cortisol ng/mL. An increase in the amount of cortisol ng/mL was accompanied by an increase in the Plaque Index (PI). With R = 0.03 and p>0.05 (p=0.834), a very weak positive, insignificant correlation was determined.

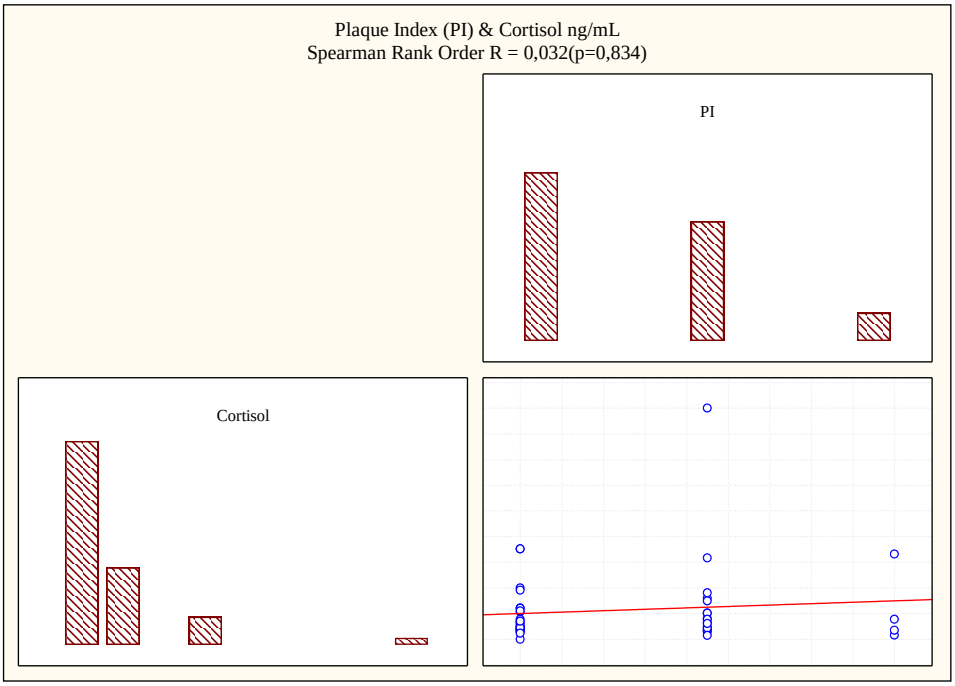

Figure 38. Correlation between Plaque Index and cortisol levels in the ASD group

Figure 39 shows the correlation between the BEWE Index and cortisol ng/mL. An increase in the amount of Cortisol ng/mL was accompanied by an increase in the BEWE Index. With R = 0.15 and p>0.05 (p=0.316), a moderately weak positive, insignificant correlation was determined.

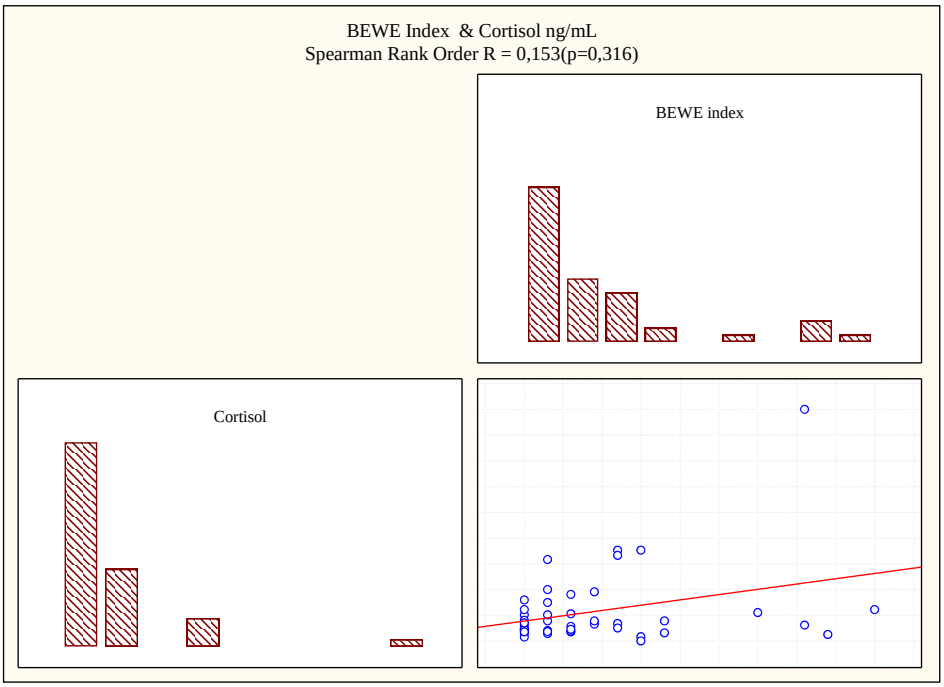

Figure 39. Correlation between BEWE Index and cortisol levels in the ASD group

Figure 40 shows the correlation between the dmft index and melatonin pg/mL. The increase in the amount of melatonin pg/mL was accompanied by a decrease in the dmft index. With R = -0.01 and p>0.05 (p=0.929), a very weak negative, insignificant correlation was determined.

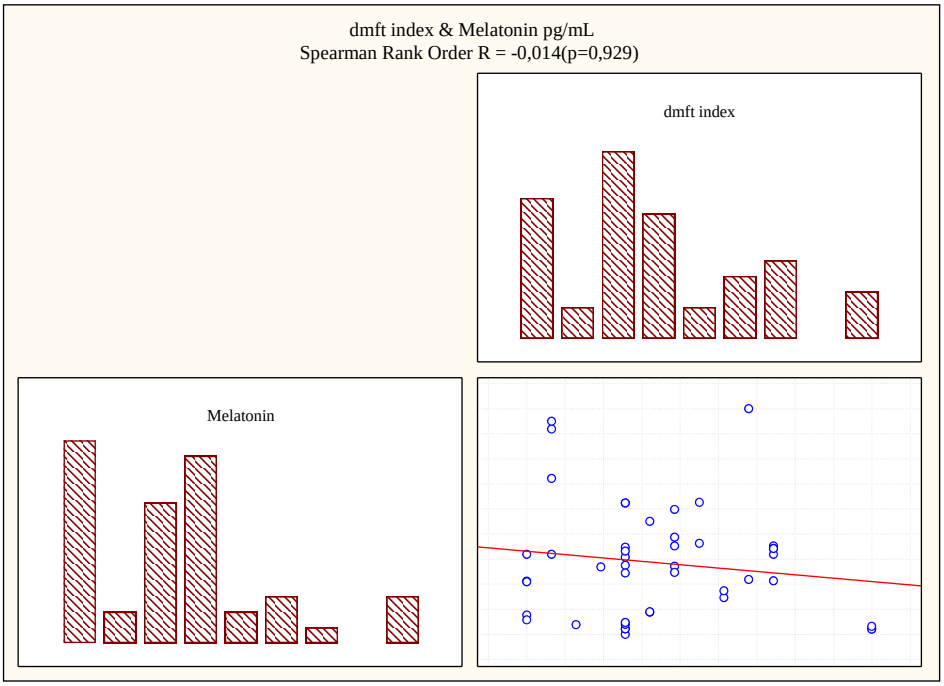

Figure 40. Correlation between dmft index and melatonin levels in the ASD group

Figure 41 shows the correlation between the Gingival Index (GI) and melatonin pg/mL. An increase in the amount of melatonin pg/mL was accompanied by a decrease in the Gingival Index (GI). With R = -0.12 and p>0.05 (p=0.541), a weak negative, insignificant correlation was determined.

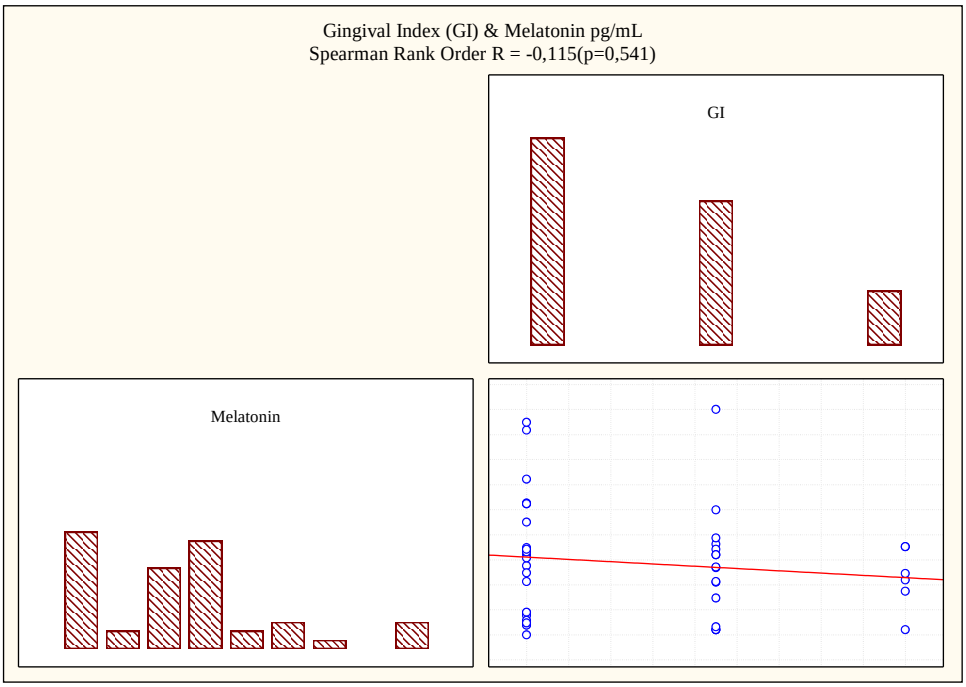

Figure 41. Correlation between Gingival Index and melatonin levels in the ASD group

Figure 42 shows the correlation between the Plaque Index (PI) and melatonin pg/mL. The increase in the amount of melatonin pg/mL was accompanied by a decrease in the Plaque Index (PI). With R = -0.19 and p>0.05 (p=0.202), a moderately weak negative, non-significant correlation was determined.

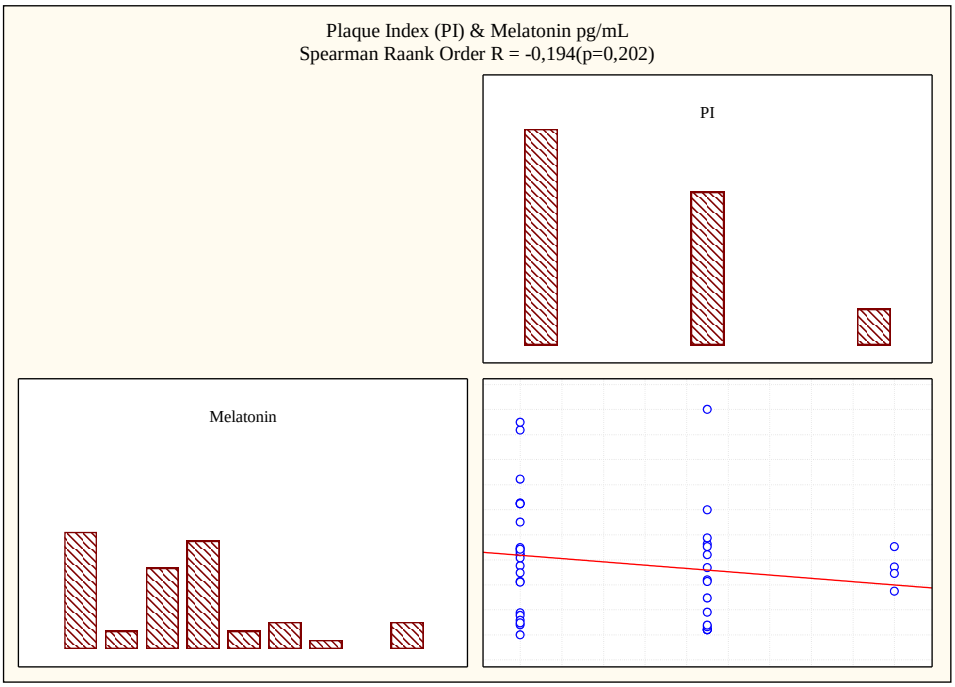

Figure 42. Correlation between Plaque Index and melatonin levels in the ASD group

Figure 43 shows the correlation between the BEWE Index and melatonin pg/mL. An increase in the amount of melatonin pg/mL was accompanied by an increase in the BEWE Index. With R = 0.19 and p>0.05 (p=0.206), a weak non-significant, correlation was determined.

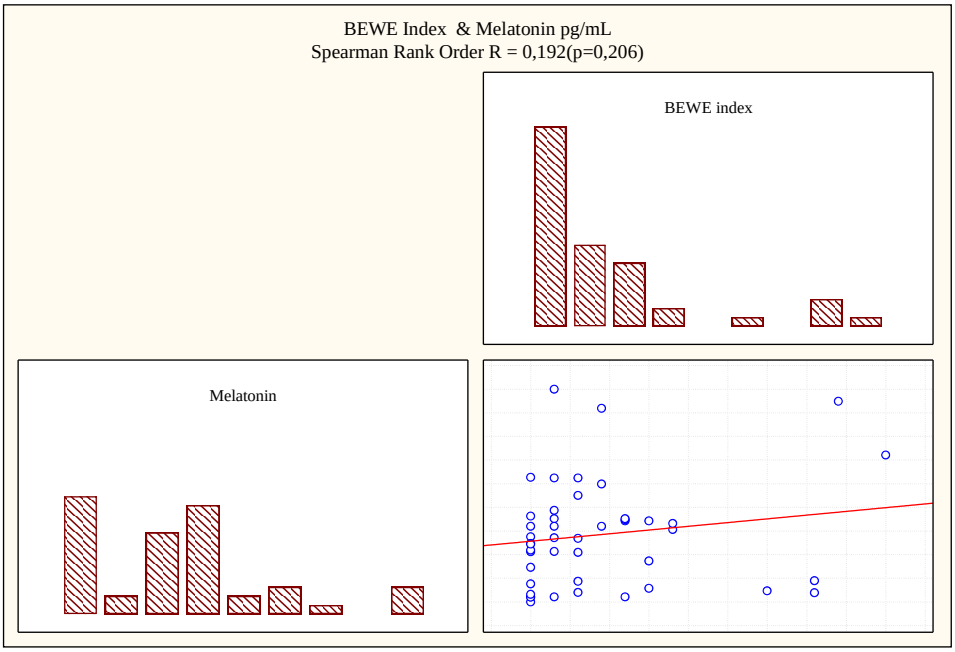

Figure 43. Correlation between BEWE Index and melatonin levels in the ASD group

Figure 44 shows the correlation between cortisol ng/mL and melatonin pg/mL. An increase in the amount of melatonin pg/mL was accompanied by an increase in the amount of cortisol ng/mL. With R = 0.20 and p>0.05 (p=0.192), a moderately weak positive, insignificant correlation was determined.

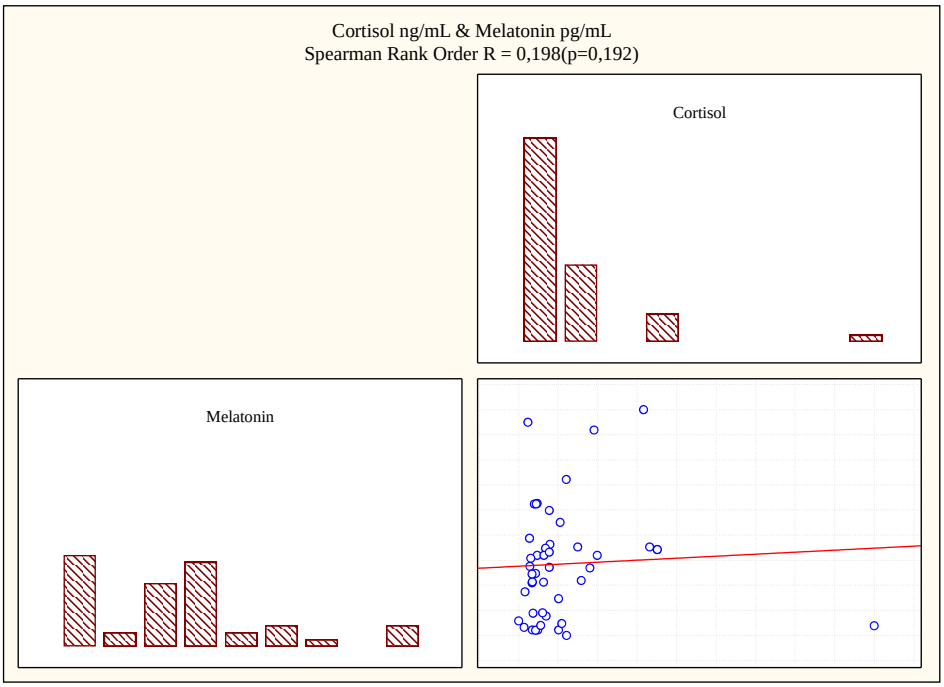

Figure 44. Correlation between cortisol and melatonin levels in the ASD group

Figure 45 shows the correlation between the Gingival Index (GI) and the dmft Index. An increase in the dmft index was accompanied by an increase in the Gingival Index (GI). With R = 0.54 and p<0.001 (p=0.000), a moderately strong, significant correlation was determined.

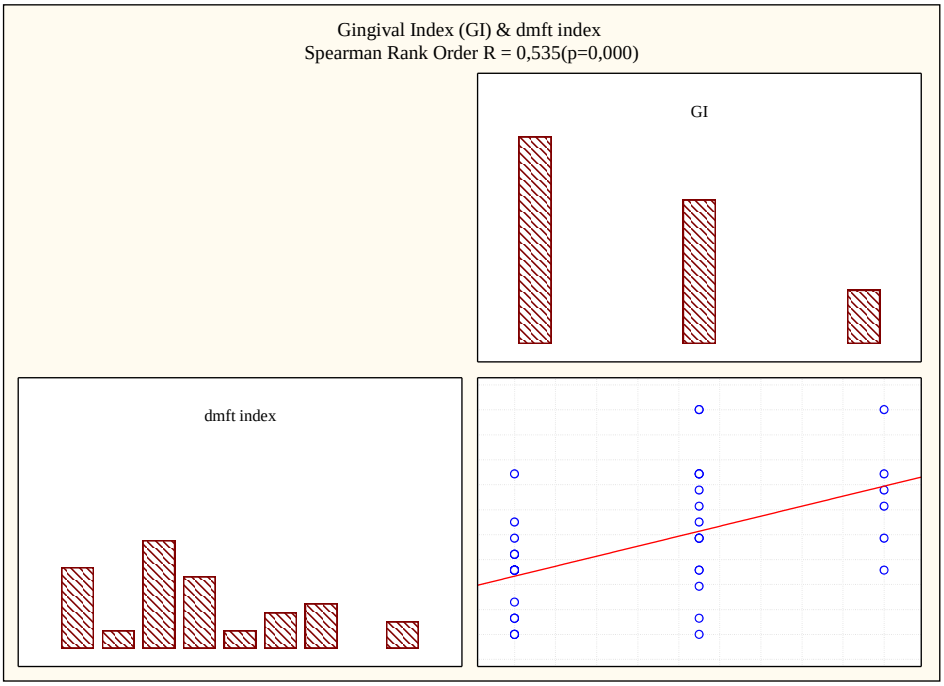

Figure 45. Correlation between Gingival Index and dmft index in the ASD group

Figure 46 shows the correlation between the BEWE index and the Plaque Index (PI). An increase in the Plaque Index (PI) was accompanied by a decrease in the BEWE index. With R = -0.13 and p>0.05 (p=0.387), a moderately weak negative, insignificant correlation was determined.

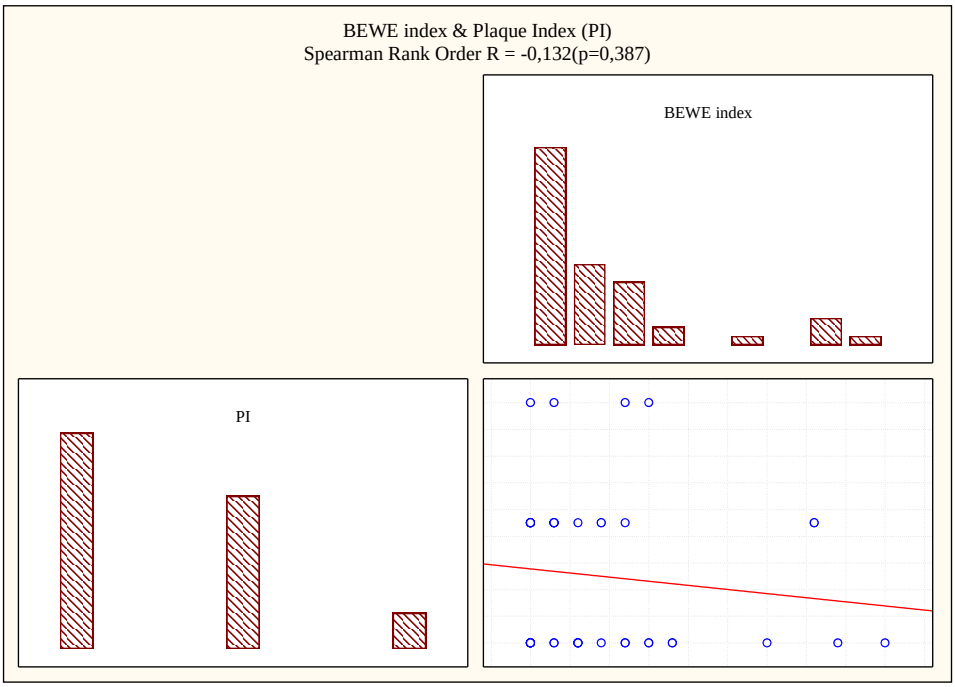

Figure 46. Correlation between BEWE Index and Plaque Index in the ASD group

4.6.2.2. Multiple regression analysis

In the ASD group, multiple linear regression analysis was performed to identify independent predictors of the dmft index. Sex, age, Plaque Index, *Candida albicans* status, *Candida albicans* colony count, and cortisol were included as independent variables. *Candida albicans* status was excluded from the model because of collinearity. The model was statistically significant, $R = 0.582$, $F = 3.998$, $p = 0.005$. Plaque Index was the only statistically significant predictor of the dmft index, $\beta = 0.437$, $p = 0.002$, indicating that higher plaque accumulation was associated with higher dmft index values. Sex, age, *Candida albicans* colony count, and cortisol were not statistically significant predictors (Table 23).

Table 23. Multiple linear regression analysis of predictors of the dmft index in the ASD group

Model	Unstandardized Coefficients		Standardized	t	Sig.	95,0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	-6.706	3.559		-1.884	0.067	-13.906	0.493
Sex (1)	1.564	1.024	0.207	1.526	0.135	-0.508	3.636
Age	1.223	0.69	0.246	1.773	0.084	-0.172	2.618
Pl	2.527	0.761	0.437	3.321	0.002	0.988	4.067
CA(CFU)	0.029	0.021	0.187	1.41	0.167	-0.013	0.072
Cortisol	-0.148	0.121	-0.177	-1.23	0.226	-0.392	0.096

a. Dependent Variable: dmft index

PI - plaque index; CA - Candida albicans; CFU - colony-forming units; dmft - decayed, missing, and filled teeth index; ASD - autism spectrum disorder.

In the second regression model, cortisol was used as the dependent variable, while age, Gingival Index, *Candida albicans* status, and melatonin were included as independent variables. The model was not statistically significant, R = 0.348, F = 1.375, p = 0.260. Although the overall model was not statistically significant, age was the only statistically significant predictor of cortisol levels, β = 0.333, p = 0.032, indicating that cortisol levels increased with age in the ASD group. Gingival Index, *Candida albicans* status, and melatonin were not statistically significant predictors (Table 24).

Table 24. Multiple linear regression analysis of predictors of cortisol levels in the ASD group

Model		Unstandardized Coefficients		Standardized	t	Sig.	95,0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
	(Constant)	-5.947	4.654		-1.278	0.209	-15.353	3.458
	Age	1.971	0.886	0.333	2.225	**0.032**	0.181	3.76
	GI	0.264	0.963	0.042	0.274	0.785	-1.683	2.211
	CA	-1.288	1.446	-0.137	-0.89	0.379	-4.211	1.635
	Melatonin	0.002	0.079	0.005	0.031	0.976	-0.157	0.162

a. Dependent Variable: Cortisol

CA - Candida albicans; PI - plaque index; dmft - decayed, missing, and filled teeth index.

In the third regression model, the BEWE Index was used as the dependent variable, while age, Plaque Index, dmft index, and cortisol were included as independent variables. The model was statistically significant, R = 0.462, F = 2.713, p = 0.043. The dmft index was the only statistically significant predictor of the BEWE Index, β = -0.335, p = 0.048, indicating that higher dmft index values were associated with lower BEWE Index values. Age, Plaque Index, and cortisol were not statistically significant predictors.

4.6.2.3. Binary logistic regression for Candida albicans

In relation to the study objective assessing *Candida albicans* prevalence and its association with hormonal and oral health parameters, binary logistic regression was performed to evaluate whether sex, age, dmft index, cortisol, and melatonin predicted the presence of *Candida albicans* in the ASD group. The model correctly classified 71.1% of cases. Specificity was 93.1%, indicating good identification of children without *Candida albicans*, while sensitivity was 31.3%, indicating limited ability to identify children with *Candida albicans* positivity (Table 25).

Table 25. Classification accuracy of the binary logistic regression model for Candida albicans in the ASD group

Observed			Predicted		
			Candida albicans		Percentage Correct
			Candida negative	Candida positive	
Step 1	CA	Candida negative	27	2	93.1
		Candida positive	11	5	31.3
	Overall Percentage				71,1

a. The cut off value is .500

CA - Candida albicans; dmft - decayed, missing, and filled teeth index.

None of the included predictors showed a statistically significant independent contribution to the prediction of *Candida albicans* presence in the ASD group. The strongest predictors were the dmft index, Wald = 1.826, p = 0.177, and melatonin, Wald = 1.452, p = 0.228; however, both were not statistically significant. Sex, age, and cortisol were also not statistically significant predictors (Table 25.1).

Table 25.1 Binary logistic regression analysis for predictors of *Candida albicans* presence in the ASD group

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Sex (1)	(.701)	.735	.909	1	.340	.496	.118	2.095
	Age	.292	.479	.372	1	.542	1.339	.524	3.422
	dmft index	.130	.096	1.826	1	.177	1.138	.943	1.373
	Cortisol	(.040)	.089	.199	1	.656	.961	.806	1.145
	Melatonin	(.055)	.045	1.452	1	.228	.947	.866	1.035
	Constant	(1.672)	2.203	.576	1	.448	.188		

a. Variable(s) entered on step 1: Sex (1), Age, dmft, Cortisol, Melatonin.

The ROC analysis showed an area under the curve of 0.688, 95% CI: 0.517–0.858, p = 0.039. This indicates acceptable overall discriminatory ability of the model for predicting the presence of *Candida albicans* in the ASD group. However, considering the low sensitivity of the model, this finding should be interpreted with caution (Figure 47).

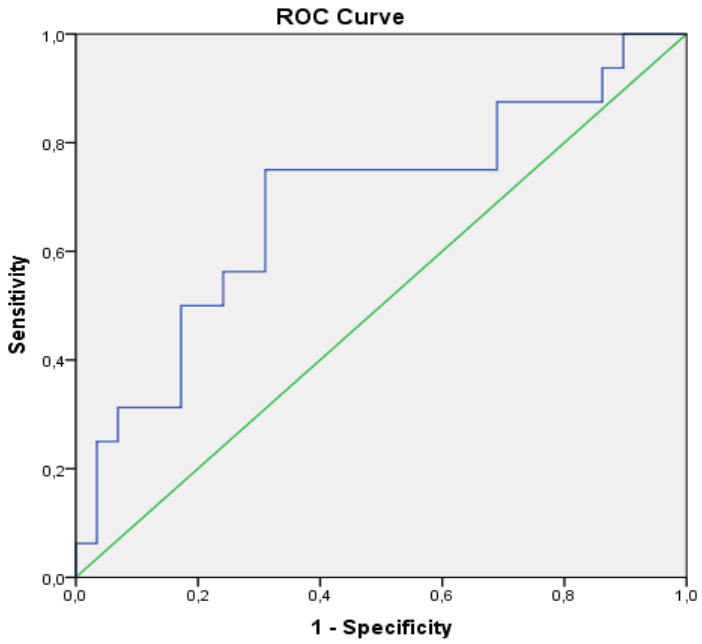

Figure 47. ROC curve for the prediction of *Candida albicans* presence in the ASD group

Overall, the binary logistic regression analysis in the ASD group showed that none of the included variables were statistically significant predictors of *Candida albicans* presence. Although the model demonstrated acceptable overall discriminatory ability based on the ROC analysis, its low sensitivity indicates limited ability to correctly identify children with *Candida albicans* positivity. Therefore, the model was more effective in identifying children without *Candida albicans* than those with *Candida albicans.*

4.6.3. Spearman correlations between study variables in the control and ASD groups

Table 26 shows the differences between the Spearman Rank Order R values in the control and ASD groups.

In the relationships: Plaque Index (PI) and *Candida albicans* (CFU/mL) / $p<0.05(p=0.045)$; dmft Index and cortisol ng/mL / $p<0.05(p=0.014)$, a significant difference was determined between the Spearman Rank Order R values in the control and ASD groups.

In the relationships: BEWE Index and *Candida albicans* (CFU/mL) / $p>0.05(p=0.097)$; Gingival Index (GI) and dmft Index / $p>0.05(p=0.068)$, a borderline insignificant difference was determined between the Spearman Rank Order R values in the control and ASD groups.

In the remaining relationships analysed for $p>0.05$, a non-significant difference was determined between the Spearman Rank Order R values in the control and ASD groups.

Table 26. Comparison of Spearman's rank correlation coefficients between the control and ASD groups

	Spearman Rank Order R		p
	Control group N=45	ASD group N=45	
dmft Index and Candida albicans (CFU/mL)	0.167	0.223	0.699
Gingival Index (GI) and Candida albicans (CFU/mL)	0.238	0.172	0.650
Plaque Index (PI) and Candida albicans (CFU/mL)	0.354	0.068	**0.045**
BEWE Index and Candida albicans (CFU/mL)	-0.178	0.160	0.097
dmft Index and Cortisol ng/mL	-0.396	0.035	**0.014**
Gingival Index (GI) and Cortisol ng/mL	-0.239	0.054	0.229
Plaque Index (PI) and Cortisol ng/mL	-0.117	0.032	0.447
BEWE Index and Cortisol ng/mL	-0.194	0.153	0.123
dmft Index and Melatonin pg/mL	-0.150	-0.014	0.478
Gingival Index (GI) and Melatonin pg/mL	-0.067	-0.115	0.717
Plaque Index (PI) and Melatonin pg/mL	-0.066	-0.194	0.327
BEWE Index and Melatonin pg/mL	0.034	0.192	0.276
Cortisol ng/mL and Melatonin pg/mL	-0.063	0.198	0.140
Gingival Index (GI) and dmft Index	0.285	0.535	0.068
BEWE Index and Plaque Index (PI)	-0.087	-0.132	0.739

* p values were obtained using Fisher r-to-z test.

5. DISCUSSION

In this doctoral dissertation, the relationship between oral health status and hormonal fluctuations, specifically cortisol and melatonin levels, was investigated in preschool children with autism spectrum disorder (ASD) aged 4–6 years, compared to their typically developing peers. This study aimed to provide a comprehensive and multidimensional perspective of the oral health of children with ASD by recording clinical oral health indices, undertaking microbiological assessment of Candida albicans, and salivary hormonal analysis.

Overall, the findings indicate that children with ASD exhibit poorer oral health outcomes and distinct biological profiles than healthy controls. These results support the hypothesis that oral health in children with ASD is influenced not only by behavioural and sensory factors but also by underlying neuroendocrine changes. The combined assessment of clinical indices, C. albicans colonisation, and salivary hormonal markers could be a valuable contribution to the existing literature, as these variables have rarely been examined simultaneously in preschool children with ASD.

This study showed that Kosovar preschoolers with ASD had poorer oral health outcomes than those in the control group. In particular, higher levels of dental caries (decayed, missing, and filled teeth [dmft] index scores), plaque accumulation (Plaque Index [PI]) and gingival inflammation (Gingival Index [GI] scores) were observed in the ASD group than in the control group; however, the difference was not statistically significant ($p = 0.54$, 0.68, 0.34, and 0.80, respectively). In addition, there was no statistically significant difference in the Basic Erosive Wear Examination (BEWE) scores between the groups (ASD: 4.98 ± 3.85 vs. controls: 3.93 ± 2.03; $p = 0.80$). Notably, in the ASD group, a strong positive association was observed between gingival inflammation and caries, highlighting the important role of local inflammatory processes in oral disease progression. These results suggest that children with ASD tend to have less favourable oral health. These findings may be attributed to the challenges faced by children with ASD in maintaining proper oral hygiene and accessing regular dental care. The consistently high dmft scores observed in children with ASD are clinically relevant. These findings suggest that caries in children with ASD may develop early and progress gradually, potentially influenced by behavioural challenges, dietary habits, and difficulties in maintaining adequate oral hygiene. Furthermore, the absence of strong statistical associations may reflect the young age of the participants, in whom the cumulative effects of oral disease have not yet been fully manifested.

Similar patterns have been documented in the literature. For example, a meta-analysis including 1,040 participants in 8 studies from various countries showed that children with ASD have higher average caries indices and plaque levels than neurotypical children (64).

However, data contradicting these findings have been previously published. For example, several studies from developed countries, including the United States, Sweden, and Japan, have reported similar or even lower caries prevalence in ASD populations than in controls. This has been attributed to factors such as specialised support services, systematic preventive care, and personalised interventions aimed at improving well-being (65-67). In contrast, studies in countries with limited access to public health services, such as India, Turkey, and Iran, have found either a higher prevalence of caries in children with ASD or no significant difference between the ASD and non-ASD groups (68-70). These discrepant findings suggest that social and health system factors influence oral health outcomes in children with ASD.

Beyond clinical indicators, our study found that dental conditions were associated with reduced OHRQoL in children with ASD. Parents in the ASD group reported significantly higher total P-CPQ-16 scores (38,71), with notable impacts on the “functional”, “social”, and “emotional well-being” domains (72). This indicates that untreated oral diseases in patients with ASD are linked to physical and emotional distress, resulting in decreased daily functioning. This aligns with the findings of Salerno et al., who demonstrated that children with ASD have significantly lower OHRQoL than their neurotypical counterparts, particularly in the functional, emotional, and social domains (72). Fallea et al. also found that individuals with ASD report a decreased overall quality of life because of poor oral health. These observations highlight that oral disease in this group is not only a medical concern but also has psychological and social implications for children and their families (73).

Our results are consistent with those reported in previous studies examining patterns of oral health behaviour and barriers to dental care in children aged 3–5 years, with and without ASD (65,74,77). Children with ASD brushed their teeth less frequently, applied less toothpaste, and were more dependent on their caregiver or parent for tooth brushing (65,77). They also experience challenges in obtaining dental care and limited access to dental services, although their parents demonstrate greater knowledge and more positive attitudes toward oral health than parents of children without ASD (74,77). In addition, children with ASD may exhibit higher tendencies toward parafunctional oral behaviours and bruxism-related behaviours, which may further complicate oral health management (75).

The main difference between these previous studies and ours was the frequency of toothpaste use in oral hygiene practices. Previous studies have found that almost all children in both the control and ASD groups used toothpaste, with no significant difference between them (65). This difference between the two studies may be attributed to increased parental awareness, cultural habits regarding oral hygiene, and the influence of local health education initiatives.

Nonetheless, similar conclusions have been drawn regarding barriers to care. In both studies, children with ASD reportedly visited the dentist less often, and their most recent visits were typically related to toothache, in contrast to the findings in the control group, where routine check-ups were the primary reason. This confirms that access to dental care for children with ASD is challenging across different cultural contexts (74).

However, a high level of awareness was not consistently associated with better oral health practices in these children, suggesting that one of the structural and functional barriers to good oral healthcare may be persistent behavioural issues, such as poor diet, ASD stereotypies, and neglect of oral hygiene.

A significantly higher proportion (86.7%) of caregivers of children with ASD reported that visiting a dentist was challenging because of the child's behaviour or sensory issues, whereas similar issues were reported for only 28.9% of the controls ($p < 0.001$). Reports on the barriers faced by those with ASD in accessing services in several countries indicate that fear, hypersensitivity to dental stimuli, and communication difficulties restrict visits to dentists, who could prevent the development of more serious conditions (37), and this results in more frequent emergency care visits. This aligns with our finding that dental visits in the ASD group were typically motivated by toothache rather than a regular check-up.

The adapted amalgamated questionnaire used in this study also revealed higher frequencies of self-injurious or maladaptive oral behaviours in children with ASD. In the present study, we found that 44.4% of children with ASD often put objects in their mouths, 33.3% exhibited bruxism/tooth grinding, and 4.4% hit or kicked their own mouth/face; the incidences for all these behaviours were significantly lower in the control participants ($p < 0.001$). These trends are in line with the findings of Winocur-Arias et al., who discovered a higher prevalence of bruxism and parafunctional habits among Israeli adolescents with ASD (75), and those of Richards et al., who reported higher rates of oral self-injurious behaviours in ASD cohorts (76). Such behaviours increase the risk of trauma, enamel wear, and toothache, further complicating daily oral care. Moreover, studies have found associations between commonly prescribed medications for ASD, such as antipsychotics, antiepileptics, and stimulants, and conditions such

as xerostomia, which, in turn, may increase the risk of dental caries (77). However, our study found low rates of use of these medications, and antiepileptic drug users were excluded. Although no significant associations between medication use and oral health outcomes were identified, this may have resulted from limited exposure rather than a lack of such effects. Nonetheless, the combination of these pharmacological effects and behavioural difficulties may explain why populations with ASD in other countries exhibit higher burdens of oral disease than control groups. The recommended strategies for preventing adverse pharmacological effects include fluoride supplements, saliva substitutes, and shorter retreatment intervals, tailored for patients receiving medications for ASD.

Cultural influences mediate the association between ASD and oral health. For example, in Iran, Piraneh et al. found that 65.0% of children with ASD brushed their teeth daily, and 73.7% used a fluoride toothpaste; the latter is a significantly higher rate than that reported in our study group (70). Therefore, culturally adapted caregiver training and oral health promotion programmes should effectively improve daily oral hygiene practices, even for those facing neurodevelopmental challenges. Research on behavioural interventions to improve self-brushing has also yielded promising results in enhancing oral hygiene independence in children with ASD (78). These results should be combined with the answers obtained for Q15–25 in the amalgamated questionnaire in this study, which suggest that various factors impede oral hygiene in ASD, including reliance on caregiver assistance, behavioural disinclination, increased sensory sensitivity during dental check-ups, and harmful oral practices. Our findings regarding the high prevalence of bruxism and oral self-injurious behaviours among children with ASD are strongly supported in the international literature, and reinforce the need for integrated approaches, including caregiver education, sensory-adapted dental settings, and preventive dental strategies involving medication.

A key finding of the present study was the higher prevalence and increased colony counts of C. albicans in children with ASD, suggesting greater susceptibility to oral microbial imbalance. This observation is consistent with previous reports indicating altered oral microbiota and increased fungal colonisation in children with ASD, which are potentially related to behavioural factors, dietary habits, and immune differences (24,32,79,80).

Although correlations between *C. albicans* counts and oral health indices were generally weak, consistent positive trends were observed. A statistically significant, moderate positive correlation was found only with the PI scores in the control group, whereas associations with dmft and GI scores were non-significant. In the ASD group, all correlations were weak and not

statistically significant, suggesting that factors other than the clinical oral health indices may influence the presence of *C. albicans* in this population.

These findings are partly consistent with those of studies reporting weak or non-significant associations between *Candida* and caries indices, such as the study by Najeeb et al. (81), whereas other studies have demonstrated stronger associations between increased fungal load and higher dmft and GI scores (47). This variability across studies may be explained by differences in age groups, study designs, and population characteristics.

Importantly, *C. albicans* is known to interact dynamically with bacterial communities within the oral biofilm, rather than acting as an independent pathogen. Fungal–bacterial interactions enhance biofilm structural complexity, increase microbial virulence, and contribute to disease progression through synergistic mechanisms involving acid production and immune modulation (26,27). In this context, the findings of the present study suggest that *C. albicans* may serve as an indicator of a biologically altered oral environment to a greater extent than as a direct causative factor of oral diseases, particularly in populations with underlying systemic or behavioural differences (26,79). The weak correlation observed between *C. albicans* and conventional oral health indices further supports this interpretation, highlighting the multifactorial nature of oral microbial dysbiosis, particularly in children with ASD (79,81). Additionally, behavioural characteristics commonly associated with ASD, including dietary preferences and difficulties in maintaining adequate oral hygiene, may contribute to an oral environment that favours microbial imbalance beyond that reflected by conventional clinical indices (78,81). The presence of *C. albicans* has also been reported in polymicrobial biofilms, where interactions with bacterial communities enhance microbial virulence and contribute to disease-related processes (27,82). In line with these findings, the results of the present study further support the idea that, even in the absence of strong statistical correlations, *C. albicans* colonisation may reflect an altered oral ecosystem, particularly in children with ASD. This may explain the weak but consistent associations observed between *C. albicans* and oral health indices.

Alterations in immune function in children with ASD may further influence host–microbe interactions, potentially facilitating microbial imbalances, including increased fungal colonisation (82,83). Consequently, traditional oral health indices, such as the dmft and GI, may not fully reflect the underlying microbial and immunological changes in this population (79).

These findings emphasise the importance of a comprehensive approach to oral health assessment that integrates microbiological evaluations with clinical indices and behavioural

factors in children with ASD. Future research employing longitudinal study designs and broader microbial profiling, with the assessment of immune and behavioural parameters, may help clarify whether increased *C. albicans* colonisation is a risk marker for future oral diseases or a reflection of systemic and behavioural factors unique to ASD (79,80).

On the basis of these findings, it is important to consider the role of systemic factors, particularly hormonal and neuroendocrine regulation, in shaping the oral environment in children with ASD. Hormones influence immune responses, salivary composition, and microbial balance, which may, in turn, affect both bacterial and fungal colonisation and overall oral health outcomes in this population.

The present study revealed differences in the hormonal profiles between children with ASD and controls, particularly in cortisol and melatonin levels. Children with ASD demonstrated significantly higher cortisol levels (mean 3.60 ± 4.54 ng/mL) than controls (mean 1.13 ± 1.07 ng/mL; $p < 0.001$), indicating an altered stress response and increased activation of the hypothalamic–pituitary–adrenal (HPA) axis. In contrast, melatonin levels were lower in children with ASD (mean 11.16 ± 8.76 pg/mL) than in controls (mean 15.27 ± 10.10 pg/mL; $p = 0.04$), suggesting potential disruptions in circadian rhythm regulation. These findings are consistent with those of previous studies reporting increased stress responses and neuroendocrine dysregulation in individuals with ASD (84,85).

Cortisol is a well-established biomarker of stress and HPA axis activity, and alterations in its regulation have been extensively documented in individuals with ASD (84,85). In the present study, cortisol levels were associated with several oral health indicators; however, most of these correlations were not statistically significant.

When examining the relationship between hormonal parameters and oral health indices, a moderate negative correlation was observed between cortisol levels and dmft scores in the control group ($r \approx -0.40$, $p \approx 0.007$), suggesting that higher cortisol levels were associated with a lower caries experience. Interestingly, this finding was further supported by regression analysis, where cortisol level emerged as a significant predictor of the dmft score in the control group, indicating a direct and quantifiable relationship between stress-related hormonal activity and oral health outcomes in typically developing children.

However, this finding may seem paradoxical and reflect indirect or compensatory mechanisms rather than a direct protective effect of cortisol. In contrast, in the ASD group, all correlations between cortisol levels and oral health indices were non-significant, indicating a lack of

consistent linear relationships between stress-related hormonal activity and oral disease measures.

Notably, differences in the relationship between cortisol levels and dmft scores between the ASD and control groups suggest a potential group-specific interaction, possibly influenced by the underlying biological and behavioural characteristics associated with ASD. Similarly, the correlations between melatonin and oral health indices were inconsistent in both groups, further supporting the absence of a direct endocrine–oral health link.

These findings suggest that hormonal influences on oral health are likely indirect and are mediated through complex physiological pathways. Stress-related mechanisms may affect oral health through immune modulation, alterations in salivary flow, and behavioural responses (77,85-87). Children with ASD often experience chronic stress related to sensory sensitivity, communication challenges, and disrupted routines, which may contribute to sustained activation of the HPA axis. Although the GI and PI scores did not show statistically significant correlations with cortisol levels, the observed trends suggest that stress-related hormonal changes may contribute to oral inflammation over time (85). Neuroendocrine dysregulation in ASD can affect multiple physiological systems, indicating that the cortisol-related effects on oral tissues may be subtle, cumulative, or not immediately detectable in early childhood (84,85). Overall, these findings reinforce the concept that hormonal changes in ASD do not act as isolated causative factors, but as components of a complex, multifactorial system influencing oral health, microbial balance, and susceptibility to disease.

Melatonin plays a central role in regulating circadian rhythms, sleep–wake cycles, and immune modulation. Reduced melatonin levels have been consistently reported in children with ASD, particularly in those with sleep disturbances and anxiety-related symptoms (84). In the present study, melatonin levels showed non-significant correlations with oral health indices, including the dmft index, GI, PI, and BEWE index.

The weak correlations observed in this study may reflect the young age of the participants, as the effects of melatonin dysregulation are likely cumulative, and may become more evident over time. Additionally, melatonin has been shown to regulate salivary secretion and immune defence mechanisms, suggesting that reduced melatonin levels in children with ASD may contribute to the development of oral dysbiosis. This is further supported by evidence demonstrating the inhibitory effects of melatonin on microbial growth and biofilm formation, including *C. albicans* (88, 89).

The present findings demonstrate that the relationships between hormonal parameters (cortisol and melatonin) and *C. albicans* colonisation were generally weak and not statistically significant in both the ASD and control groups.

In the control group, a weak positive correlation was observed between cortisol levels and *C. albicans* counts ($R = 0.09$, $p > 0.05$), suggesting a slight tendency towards increased fungal colonisation with higher cortisol levels; however, this association was not statistically significant. Similarly, melatonin showed a weak positive correlation with *C. albicans* counts ($R = 0.03$, $p > 0.05$), indicating no significant relationship.

In the ASD group, cortisol also demonstrated a negligible positive correlation with C. albicans counts ($R = 0.01$, $p > 0.05$), confirming the absence of a significant association between stress-related hormonal activity and fungal colonisation. In contrast, melatonin showed a weak negative correlation ($R = -0.15$, $p > 0.05$), suggesting a potential inhibitory trend, although this finding was not statistically significant.

No significant differences were observed between the ASD and control groups, indicating that the relationship between hormone levels and *C. albicans* colonisation did not differ substantially between the two populations. Overall, these results suggest that cortisol and melatonin are not direct determinants of fungal colonisation. Instead, their influence appears to be indirect and is likely mediated through immune modulation, salivary composition, and host–microbial interactions.

This interpretation is consistent with previous studies reporting that hormonal effects on microbial colonisation are complex and often mediated through intricate biological mechanisms rather than direct antimicrobial action (88,89). In particular, melatonin has been shown to possess antifungal, antioxidant, and immunomodulatory properties, which may help regulate microbial balance rather than directly determining colonisation levels.

To further investigate the role of hormonal factors, a binary logistic regression analysis was conducted. The results indicated that neither cortisol nor melatonin were statistically significant predictors of the presence of *C. albicans* in either group. However, in the ASD group, higher melatonin levels were associated with a reduced likelihood of colonisation, suggesting a potential protective effect, although the effect was not statistically significant.

Similar trends have been observed in previous studies, which linked melatonin to reduced fungal growth and improved immune defence, particularly under conditions characterised by biological imbalance (89). The absence of statistical significance in the results of the present

study may be attributed to sample size limitations and variability in hormone levels among young children.

The regression models demonstrated an acceptable overall performance, particularly in the control group, indicating that age, dmft score, and hormonal parameters may collectively contribute to the presence of *C. albicans*, even if individual predictors are not independently significant. These findings further support the concept that oral fungal colonisation is multifactorial and influenced by various interacting biological and behavioural factors.

As has been reported in the literature on ASD, hormonal dysregulation affects multiple physiological systems, including immune and oral health processes (77,82,84,85). However, as observed in this study, such hormonal alterations seem to function as modifying factors rather than direct causal determinants of oral microbial colonisation.

The multiple and logistic regression analyses conducted in this study provided additional insights into the multifactorial nature of oral health outcomes in children with ASD. Among the examined variables, the PI score emerged as the strongest and most significant predictor of caries (dmft score), whereas age and other factors showed weaker and non-significant contributions. In contrast, hormonal parameters such as cortisol and melatonin demonstrated limited and non-significant predictive values.

These findings suggest that hormonal dysregulation alone is insufficient to account for the oral health disparities in children with ASD. Instead, cortisol and melatonin appear to act as modulatory factors that interact with behavioural patterns, immune responses, and oral hygiene practices rather than serving as independent predictors of oral disease. Similar interpretations have been reported in studies examining biological markers in paediatric populations with neurodevelopmental disorders (77,82,85).

Importantly, the acceptable accuracy of the predictive models indicates that combining clinical, biological, and demographic variables may improve the assessment of oral disease risk in children with ASD. This integrated approach highlights the importance of considering both local oral and systemic factors, rather than relying solely on individual parameters.

Beyond biological mechanisms, the present findings must be interpreted within the context of the behavioural and sensory characteristics associated with ASD. Sensory hypersensitivity, resistance to tooth brushing, and communication difficulties can significantly impair daily oral hygiene routines. Al-Beltagi et al. and Sarvas et al. have emphasised that these challenges often lead to delayed dental visits and limited preventive care (77,90).

In line with our findings, which show that majority of children with ASD required assistance during tooth brushing, the international literature reports similar challenges in home-based oral care. A substantial proportion of children with ASD experience difficulties during tooth brushing and may resist parental assistance, primarily because of sensory sensitivity and behavioural challenges (39,40). These findings support the interpretation that sensory and behavioural factors play a significant role in the independence and cooperation of children with ASD in maintaining oral hygiene.

Parental involvement plays a crucial role in shaping oral health outcomes in children with ASD. Goursand et al. demonstrated that parental perceptions of oral health strongly influenced care-seeking behaviour and quality of life (71,81,90). Our study indirectly supports these findings by highlighting that oral health disparities emerge early in life, underscoring the importance of caregiver education and the tailoring of preventive strategies.

Interventional studies have shown that structured behavioural approaches, particularly those involving parents, can significantly improve oral hygiene in children with ASD (78). The results of this study provide further justification for implementing such interventions at preschool age before oral disease becomes more severe.

In our study, 64.4% of the children required assistance during tooth brushing. Similarly, Alqahtani et al. reported that 88% of parents assisted their children with tooth brushing (91). They also found that only 22% of parents used dental floss for their children and that most dental visits were scheduled primarily in response to pain rather than during preventive check-ups. A comparison of these results with our data highlights the similarities and differences in parental involvement, flossing practices, and patterns of dental attendance among children with ASD.

Not all populations of children with ASD demonstrate a low frequency of tooth brushing. Hassan et al. reported that approximately 44% of children with ASD brushed their teeth twice daily (92). This indicates that suboptimal brushing frequency is not a universal finding among children with ASD. These differences may be influenced by cultural factors, parental education, and the organisation of the healthcare system.

Regarding dental attendance, Hassan et al. reported that 23% of children with ASD attended regular dental visits, which is comparable to the 24.4% observed in the present study (92). This similarity suggests a consistent pattern of limited utilisation of preventive dental care among children with ASD, despite geographical differences.

The findings of this study have important clinical implications. First, they highlight the importance of early oral health screening in children with Autism Spectrum Disorder (ASD), focusing on oral hygiene, plaque control, gingival health, and fungal colonization. Second, the hormonal changes that occur suggest that oral health professionals should consider broader physiological and behavioural factors when managing children with ASD. Personalized oral health approaches that address sensory sensitivity, stress levels, and sleep difficulties may be more effective than conventional approaches. For example, they suggest the potential value of preventive measures for patients with ASD, such as intensified preventive efforts with more regular check-ups every 3–4 months, regular application of fluoride varnishes, and the use of silver diamine fluoride for asymptomatic lesions when the patient is unwilling to cooperate. Caregivers of children with ASD should be adequately trained, including in task analysis, gradual desensitisation, and the use of timers and visual cues to reduce reliance on others and increase the efficiency of tooth brushing. Moreover, clinically adapted sensory flows (a calming environment, short waiting times, and a predictable schedule) that can encourage dental attendance for routine check-ups may be beneficial for improving the oral health of patients with ASD. These points directly address the weaknesses identified by the questionnaire and are consistent with the recommendations for preschoolers with ASD (90). A major strength of this study is its multidimensional design, which integrated clinical, microbiological, and hormonal assessments of a well-defined preschool population. This approach provides a more comprehensive understanding of oral health in children with ASD than studies that focus on a single domain.

However, this study had certain limitations. The cross-sectional design limits causal inference, and the relatively small sample size may have reduced statistical power. Additionally, external factors such as diet, sleep quality, and socioeconomic status were not fully controlled for, which may have influenced the results.

Future research should use longitudinal designs to explore the effects of hormonal dysregulation and oral health outcomes in children with ASD over time. Larger sample sizes and the inclusion of additional biomarkers may further clarify the biological mechanisms underlying the risk of oral diseases in this population.

Taken together, the findings of this study suggest that oral health disparities in children with ASD result from an interplay of behavioural challenges, altered oral environments, and neuroendocrine imbalances, rather than a single causative factor. This multidimensional

perspective underscores the need for early personalised preventive strategies for this vulnerable population.

6. CONCLUSION

This study sheds light on the relationship between oral status, *Candida albicans* colonization, and hormonal levels in Kosovar preschool children with autism spectrum disorder (ASD) compared to their neurotypical peers. Its design combined clinical assessments (dmft index, plaque index, gingival index, and BEWE), laboratory analysis of *C. albicans* colonization, and salivary cortisol and melatonin measurements, providing a snapshot of the interaction between oral health and the neuroendocrine system.

In relation to the first objective, significant differences in hormonal levels were observed between the groups. Children with ASD had higher cortisol levels and lower melatonin levels compared with children in the control group. These findings suggest that stress-related and sleep-related physiological processes may be relevant in children with ASD. However, the associations between hormonal levels and oral health parameters were not consistent across all analyses and should therefore be interpreted with caution.

In relation to the second objective, clinical oral health parameters, including dmft index, Plaque Index, Gingival Index, and BEWE Index, did not differ significantly between the ASD and control groups. Nevertheless, children with ASD showed a tendency toward higher caries experience and gingival inflammation. Within the ASD group, a significant positive relationship was observed between dmft index and Gingival Index, suggesting that caries experience and gingival inflammation may be closely related in this population. *Candida albicans* colonization was significantly higher in children with ASD. However, *Candida albicans* was not consistently identified as an independent statistically significant predictor in regression analyses; therefore, its role should be interpreted as a potential contributing factor rather than a direct causative factor.

In relation to the third objective, behavioural and family-related factors appeared to play an important role in oral hygiene maintenance among children with ASD. Sensory sensitivity, communication difficulties, the need for assistance during toothbrushing, and difficulties during dental visits may create additional barriers to adequate oral hygiene and regular dental care. Parents and caregivers also reported a greater impact of oral health on daily functioning, emotional well-being, and social interactions among children with ASD.

Overall, the findings indicate that oral health outcomes in children with ASD are influenced by multiple interacting factors, including behavioural challenges, oral hygiene difficulties,

Candida albicans colonization, and neuroendocrine parameters. The study supports a multifactorial interpretation rather than identifying a single causative factor. These results emphasize the need for individualized preventive strategies, parent- and caregiver-focused oral health education, visual support tools, gradual desensitization approaches, and adapted dental treatment planning. Future longitudinal studies with larger samples are needed to confirm these findings and to support the development of individualized oral care guidelines for children with ASD.

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8. CURRICULUM VITAE

Argjira Veseli was born in Gjilan, Kosovo, on October 3, 1990. She earned her Doctor of Dentistry degree at the University of Prishtina in 2016, graduating with an excellent average grade of 9.64 and being recognized as an outstanding student.

Following her graduation, Argjira pursued her specialization in the Periodontology and Oral Medicine programme at the University Clinical Dental Centre of Pristina. She successfully completed it in 2024. During her residency, she gained invaluable hands-on experience dealing with diverse periodontal cases, emphasizing the importance of preventive care and patient education.

In addition to her clinical duties, Argjira demonstrated her commitment to education by serving as a Teaching Assistant at the University of Prishtina "Hasan Prishtina" Medical Faculty in the Branch of Dentistry from 2021. In this role, she supported undergraduate students in their learning journey, providing guidance in both theoretical and practical aspects of dentistry. She later advanced to Lecturer at AMECC Rezonanca, where she contributes to the education of dental students in subjects such as periodontology, oral medicine, and laser therapy.

In addition to her academic and clinical work, her research focuses on oral health, with a particular interest in the relationship between systemic and oral conditions, as well as innovative, technology-based approaches in dentistry.

Argjira has contributed to several scientific publications in international journals, covering topics such as periodontal health, oral care in patients with special needs, and contemporary diagnostic methods in dentistry.

Her professional interests include periodontology, oral medicine, periodontal disease prevention, the management of oral conditions, and the integration of advanced technologies and robotics in dental practice. She is passionate about improving patient experiences and outcomes through innovative dental practices and education.

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