

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2013 ISBN 1595-689X VOL 14(2) 2013
AJCEM/21311 -<http://www.ajol.info/journals/ajcem>
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AFR. J. CLN. EXPER. MICROBIOL 14(2): 56-61 <http://dx.doi.org/10.4314/ajcem.v14i2.3>

STUDY OF ORAL AND GINGIVAL MICROBIAL FLORA IN INSTITUTIONALIZED MENTALLY RETARDED PATIENTS OF SARI- 2011

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ABSTRACT

Introduction and Objectives: Mental retardation (MR) is a generalized disorder appearing before adulthood, characterized by significantly impaired cognitive function and deficits in two or more adaptive behaviors. The prevalence and severity of dental caries, gingivitis and periodontitis is high in patients with mental retardation. This shift to a diseased state may lead to the experience of a high mortality from septicemia, sepsis, pneumonia and endocarditis. Our purpose was to study oral and gingival microbial flora in institutionalized mentally retarded patients of Sari and to estimate D% (percentage with untreated decayed teeth) and DMFT% (percentage of population affected with dental caries)

Materials and Methods: This study was a descriptive cross-sectional type in which Plaque samples were collected from the mouth and gingiva of 138 institutionalized mentally retarded patients of Sari to culture in specific media to identify the microorganisms. In this study anaerobic bacteria were not isolated because the instrument was not available in the laboratory. The information has been analyzed by X2 T-test methods by SPSS 17 software.

Results: The isolated microorganisms were: pneumococci S(37.7%); Streptococci sp(18.8%); E.coli (16.7%); Staphylococcus(1.4%); Neisseria sp(45/6%); Salmonella(8.7%); Proteus(3.6%); Diptheroid (4.2%); Pseudomonas(0.7%). The percentage of resistant strains was found to be highest with penicillin(67.9%) and lowest with vancomycin(11%).

Conclusion: D% between all the patients were (66.66%) . Bacterial flora in mentally retarded patients were significantly higher in frequency than in normal persons. With improvement in oral health care, we can decrease these undesirable changes.

Key words: Oral and gingival microbial flora, Mental retardation, D%, Sari

INTRODUCTION

Mental retardation (MR) is a generalized disorder, which appears before adulthood and is characterized by impairment in cognitive ability and deficits in two or more adaptive behaviors (1). The prevalence and severity of dental caries, gingivitis and periodontal infection are high in patients with mental retardation. Surfaces of teeth and gum are colonized by a dense population of microbes, called plaques, which is the main factor for dental caries. Considering that dental caries and gingival diseases occur earlier in mentally retarded patients than in normal people, it is more prevalent in these patients due

to their mental and physical disabilities and inability to effectively brush their teeth and properly and regularly use mouthwashes (2-3-4). In addition to these factors, mental disabilities and motor skill disorders such as oral breathing, lip seal, the diet causing dental caries, difficulty in chewing and swallowing, abnormal stretch of face muscles, reduction of saliva flow and effects of medicines should be mentioned (2). Washing these people's teeth by nurses also involves many problems and patients have little collaboration in this regard (5). Thus, conducting this study seems necessary for physically disabled people.

Normal oral flora (the flora with proper hygiene in the mouth) can be considerably changed. Weak hygiene of the mouth leads to increased microorganisms and their pathological types in oral flora. This shift toward sickness increases mortality due to septicemia, pneumonia and endocarditis (5). In addition to the mentioned problems, it has been reported for mentally retarded patients that their bacterial resistance of mouth to the conventional antibiotics used in dentistry is different from that of normal people (6).

Different studies have been conducted to study orodental hygienic condition and prevalence of dental caries, gingivitis and periodontal infection in mentally retarded people (7&8). Different studies have been conducted to investigate their microbial flora of gingival and dental plaques and its difference from that of healthy people along with its relationship with respiratory infections. Some other studies have been done to compare antibiotic sensitivity in highly prevalent microorganisms in dental caries of mentally retarded people with that in healthy people. In the present research, for the first time, orodental hygienic condition and prevalence rate of dental caries were studied in mentally retarded people in rehabilitation centers in the city of Sari and oral bacterial flora of these people was investigated.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in 2011 on mentally retarded patients in rehabilitation centers in the city of Sari. Sampling of mentally retarded people in these centers was random. Considering the fact that the present study was conducted for the first time, around 50% (138 people) were statistically sampled. The data were recorded in the related questionnaire, which included questions on their demographics such as gender, age and education and those for their parents and some questions about their orodental hygiene condition such as daily tooth brushing, dental caries, tooth extraction, mouth ulcers and history of oral surgery, all of which were nominal qualitative variables. Questionnaires were filled by research team members while examining the patients, by the assistance of the center authorities using the data recorded in the centers, and by the cooperation of the dentist of the related center.

The samples were directly taken from the mouth (and gum) of the participants using a sterile swab, placed in liquid transport medium (Amies) and transferred to the laboratory of Sari Faculty of Medicine. After the samples were transferred to the laboratory, they were cultured in EMB and blood Agar media. Then colonies were gram stained after 24 h of incubation at 37°C; in case the isolated bacteria were gram positive cocci, catalase test was performed on them. The positive catalase colonies were identified as staphylococcus and coagulase test was performed on them. In case of positive coagulase test, the related colony was identified as staphylococcus aureus and, in case of negative test, novobiocin disc was placed. The colonies sensitive to novobiocin were recognized as staphylococcus epidermidis and resistant colonies were considered staphylococcus saprophyticus. For negative catalase colonies which indicated their streptococcus property, hemolytic test was performed. For the colonies with alpha type hemolysis, optochin disc was used and the colonies sensitive to optochin were identified as pneumococcus while resistant ones were recognized as viridans streptococcus. For beta type hemolytic colonies, bacitracin disc and SXT were used. The colonies sensitive to bacitracin and SXT resistant colonies were identified as streptococcus pyogenes while SXT and bacitracin resistant colonies were identified as streptococcus sp. In case the isolated bacteria were gram negative bacillus, they were cultured on special enterobacteriaceae media and species of the considered bacteria was identified after comparison with the enterobacteriaceae chart. One of the limitations of this project was failure to study obligate anaerobes.

Resistance and sensitivity of the isolated bacteria to ampicillin, amoxicillin, garamycin, penicillin, vancomycin and trimethoprim sulfamethoxazole were investigated for gram positive bacteria. The same was examined using cefixim, ceftriaxon, tetracycline, jentamycin, trimethoprim-sulfamethoxazole, ciprofloxacin and cefazolin for gram negative bacteria. Antibiogram was performed in Moller Hinton medium using Kirby-Bauer method according to 0.05 McFarland standards. The available data were analyzed in SPSS software (version 17) using Chi square test and ANOVA.

RESULTS

In this study, out of 138 mentally retarded patients (48 patients) in the city of Sari, 31.4% were in Pardis Center and 21.7% (30 patients)

were in each of Mehregan, Sina and Ferdowsi Centers. The studied people were classified into three age groups of below 20, 20 to 40 and above 40. In this study, $P < 0.05$ was considered significant. The studied people included 68.8 % (95 patients) females and 31.2% (43 patients) males. Mean and standard deviation of the studied people's age were 28.28 and 12.05, respectively. 4.3% (6 patients) were literate and 95.7% (132 patients) were illiterate.

36.2% (50 patients) used toothbrush and 63.8% (88 patients) did not use it. 65.9% (135 patients) had dental caries and 34.1% (3 patients) did not. 50.97% (70 patients) had a history of tooth extraction but 49.3% (68 patients) had no record. 10.979% (15 patients) had mouth ulcers but 89.1% (123 patients) did not. 5.1 % (7 patients) had record of oral surgery while 94.9% (131 patients) had no such records. 13.18% (19 patients) used antibiotics every day but 86.2% (119 patients) did not. D index was estimated in three age groups in all of the studied patients, which was as follows: below 20 (54%), 20 to 40 (76.2%), above 40 (73.3%), total (69.5%). Based on Chi square statistical test, there was a significant relationship between dental caries and age ($\chi^2 = 6.394$, $df = 2$, $Pvalue = 0.041$).

Frequency distribution of isolated bacteria was calculated from the mouth samples of the mentally retarded patients in this study as follows:

Pneumococcus 37.7% (52 patients), E.coli 16.7% (23 patients), niseriasp 45.6% (63 patients), streptococci sp 18.8% (26 patients), staphylococci sp 1.4% (2 patients), pseudomonas 0.7% (1 patient), salmonella 8.69% (12 patients), proteus 3.6% (5 patients), diphtheroid 4.2% (6 patients).

In the performed study based on Chi-square test, there was a significant relationship between frequency of isolated bacteria from the mouth samples of the studied mentally retarded patients and the following cases were found: rehabilitation centers ($p=0.000$, $df=45$), gender of patients ($p=0.000$ and $df=15$), using toothbrush ($p=0.25$ and $df=15$), dental caries ($p=0.03$ and $df=15$), tooth extraction ($p=0.009$ and $df=15$), mouth ulcers ($p=0.05$ and $df=15$) and daily antibiotic use ($p=0.01$ and $df=15$); however, it had no significant relationship with oral surgery ($p=5$, $df=15$).

Antibiotic resistance to the tested antibiotics on gram positive bacteria was as follows: ampicillin

18.5% (15 patients), amoxicillin 50.6% (41 patients), jentamycin 12.3% (10 patients), penicillin 67.9% (55 patients), vancomycin 11% (9 patients), trimethoprim-sulfamethoxazole 65.4% (53 patients).

The antibiotic profile for gram negative bacteria was as follows: gentamycin 0%, tetracyclin 2.6% (1 patient), ceftazidime 13.1% (5 patients), ciprofloxacin 0%, ceftriaxone 5.3% (2 patients), cephotaxin 42.1% (16 patients), cefixim 36.8% (14 patients), trimethoprim-sulfamethoxazole 5.3% (2 patients).

Based on Chi-square test ($p=0.00$ and $df=60$), there was a significant relationship between resistance to the tested antibiotics on gram positive and negative bacteria and the rehabilitation centers. Based on ANOVA statistical test, there was a significant relationship between age groups and frequency of isolated bacteria ($p=0.009$) and antibiotic resistance ($p=0.001$).

DISCUSSION

The present research showed that the prevalence of dental caries among 138 mentally retarded patients in Sari rehabilitation centers (D %) was 69.5%. The estimated D % was higher than the value reported by Marcia H. Tanaka et al. (2009) in Brazil [2], in which D % was equal to 0%. In the performed statistical study in this research, D% was about 63% in the age group of 7-12 years old, which was lower than the one reported by Alghahtani and Vien (2004) in Saudi Arabia [3], reported as 100%. In this study, D % was about 70% in the age group of 3-30 years old, which was in line with the one reported by Rao et al. (2001) in India (18) as 71.5%.

In this study, the prevalence of pneumococcus among mentally retarded people was 37.7%, which was higher than its prevalence in oral flora of normal people (25%) according to the International Reference (15).

In this study, the prevalence of streptococcus (sp) among mentally retarded people was 18.8%, which was higher than its prevalence reported by W. J. Losche et al. (1972) (8) as 37.8% and the rate reported by Salaco (2007) in Kuwait (6) as 39%. The estimated prevalence of streptococcus mutans in oral flora of the mentally retarded patients in this research was not significantly different from its prevalence in oral flora of the normal people in the study by Salaco (6).

FIGURE 1: FREQUENCY OF ISOLATION OF DIFFERENT BACTERIA FROM ORAL SAMPLES

BACTERIAL ISOLATE	USE OF ANTIBIOTICS		TOTAL (%)
	YES (%)	NO (%)	
Pneumococci	6 (11.5%)	46 (88.5%)	52 (100%)
E.coli	16 (69.6%)	7 (30.4%)	23 (100%)
Neisseria spp.	13 (30.6%)	50 (79.4%)	63 (100%)
Streptococci	7 (27%)	19 (73%)	26 (100%)
Staphylococci	0 (0%)	2 (100%)	2 (100%)
Pseudomonas	1 (100%)	0 (0%)	1 (100%)
Fungi	3 (37.5%)	5 (62.5%)	8 (100%)
Diphtheria	3 (50%)	3 (50%)	6 (100%)
Salmonella spp.	0 (0%)	12 (100%)	12 (100%)
Proteus spp.	0 (0%)	5 (100%)	5 (100%)

For the difference to be significant, p=0.01 Df=15

According to the International Reference, prevalence rate of this microorganism in normal floral of the normal people was 100% (15).

In this research, prevalence rate of E.coli was 16.7% and that of proteus was 3.6% while studying enterobacteriaceae family and the prevalence rate of these bacteria was higher in normal flora of the normal people, each of whom had 25% of normal flora. Prevalence rate of salmonella was 8.7% which did not exist in normal people's mouth flora and this important fact indicated fecal-oral contamination due to low hygienic level in these people.

Prevalence rate of Neisseria was 44% in this study. In normal people's flora, the prevalence of Neisseria sp and Neisseria meningitidis was 25% (15). This increased rate which was found in the present study was due to lack of separation of its different species.

Prevalence of pseudomonas aeruginosa in the present research was 0.7% which was equal to its

rate in normal oral flora of normal people (<5%) (15).

Prevalence of staphylococci sp in this study was 1.4%; this rate was about 100% in normal oral floral of normal people in terms of staphylococcus epidermidis (15). Also, it was 25% for staphylococcus aureus (15).

The prevalence rate of diphtheroids in this study was 3.5%, which was less than its prevalence rate in normal oral flora of normal people as 25% (15).

During statistical studies in this research, antibiotic sensitivity and resistance of gram positive cocci to six antibiotics of amoxicillin, ampicillin, jentamycin, penicillin, vancomycin and trimethoprim-sulfamethoxazole were studied which were 50.6%, 18.5%, 12.3%, 67.9%, 10% and 65.4%, respectively. The highest and lowest resistance was to penicillin (67.9%) and vancomycin (11%), respectively. In addition, in the study by Salaco et al. in Kuwait, the highest

resistance was to amoxicillin (43%), which was lower than that obtained in this study in two groups of healthy and mentally retarded people and the lowest resistance was to vancomycin (12%), which was in line with the rate obtained in the present work.

The present research showed that oral and gingival bacterial flora of the studied mentally retarded people was significantly difference from oral bacterial flora in the normal people (according to the recorded scale in International References), which is justified considering their clinical differences and their inability to maintain their oral hygiene.

Higher prevalence of pneumococcus in these people than that in normal people increases risk of bacterial respiratory infections and dental plaques such as salmonella, which are never

found in oral flora of normal people, were found in these people due to their oral-fecal contamination, both of the mentioned plaques could cause severe infections, especially in case of immunodeficiency. In this study, prevalence of bacterial oral flora had a significant relationship with oral hygiene of these people.

Acknowledgements

We appreciate the esteemed research deputy of University and Faculty of Medicine for financing this project. We also appreciate esteemed authorities of four rehabilitation centers for mentally retarded (Pardis, Sina, Ferdowsi and Mehregan) in the city of Sari for their sincere cooperation.

REFERENCES

1. Marcia H. Tanaka, Karina Bocardia, Katha Yukari kishimoto, Paula Jacques, Denise Madalena Palomari Spolidorio, Elisa Maria Aparecida Giro .DMFT index assessment and microbiological analysis of streptococcus mutans in institutionalized patients with special needs. *Braz J Oral Sci.* 2009;8(1):9-13
2. Al-Qahtani Z, wyne AH. Caries experience and oral hygiene status of blind, deaf and mentally retarded female children in Riyadh, Saudi Arabia (abs). *Odontostomatol Trop.* 2004; 27(105): 37-40
3. Oral hygiene, gingival and periodontal status in Down's syndrome. For the degree of master of dental surgery, The Tamilandu Dr.M.G.R. medical university. February 2005.
4. James Day, , Michael D. Martin, Mae Chin. Efficacy of a sonic toothbrush for plaque removal by caregivers in a special needs population. *SCD Special Care in Dentistry.* 1998; 18 (5).
5. Salako NO, Rotimi V, Philip L, et al. The prevalence and antibiotic sensitivity of oral Viridans streptococci in healthy children and children with disabilities in Kuwait. *Spec Care Dentist.* 2007 Mar-Apr; 27(2):67-72.
6. Howard K. Kuramitsu, Xuesong He & Renate Lux. Interspecies Interactions within Oral Microbial Communities. *Microbiology and Molecular Biology Reviews.* December 2007; 71(4) : 653-670.
7. W.J. Loesche, R.N. Hockett and S.A. Syed. The predominant cultivable flora of tooth surface plaque removed from institutionalized subjects. *Archs Oral Biol.* 1972; 17:1311-1325.
8. Jack D. Rosenberg. Gingivitis. Gum disease; Periodontal disease. *PubMed Health,* Last reviewed: February 22, 2010
9. J. M. Hardie & G. H. Bowden. Bacterhal flora of dental plaque. *Br. Med. Bull.* 1975;. 31 (2). available at bmb.oxfordjournals.org
10. S Saini, Aparna, N gupta, et al. Microbial flora in orodental infections. *Indian Journal of Medical Microbiology.* 2003; 21(2):111-114.
11. P. Gabre, M Wikstrom, T. Martinsson and L. Gahnberg. Move of Adults with Mental Retardation from Institutions to Community-based Living: Changes in the Oral Microbiological Flora. *J Dent Res.* 2001; 80(2):421-426.
12. Cláudio Mendes Pannuti¹, Roberto Fraga Moreira Lotufo¹, Silvana Cai, Maria da Conceição Saraiva, Nívea Maria de Freitas, Danilo Falsi- Effect of a 0.5% chlorhexidine gel on dental plaque superinfecting microorganisms in mentally handicapped patients. *Pesqui. Odontol. Bras.* July/Sept 2003, 17(3) .
13. Binkley, C.J., Haugh, G.S., Kitchens, D.H. Oral microbial and respiratory status of persons with mental retardation/intellectual and developmental disability: an observational cohort study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology.* 2009, 108 (5) :722-731.
14. Rao D, Hegde A M b, Munshl A K c and Mangalore. Caries prevalence amongst handicapped children of South Canara district, Karnataka. (*J Indian Sot Pedo Prev Dent* 2001; 19:2:67-73)
15. Kenneth Todar's (2011). The normal bacterial flora of humans. In: <http://www.textbookofbacteriology.net/normaflora>.