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DENTAL CALCULUS AS A SOURCE OF BACTERIAL DNA ANALYSIS IN PREHISTORIC HUMANS: A SYSTEMATIC LITERATURE REVIEWS

Potensi Kalkulus Gigi Sebagai Sumber DNA Bakteri pada Manusia Purba: Dengan Pendekatan Studi Pustaka

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Abstrak

Kalkulus gigi didefinisikan sebagai plak gigi termineralisasi yang terbentuk melalui interaksi kompleks antara saliva dan bakteri yang melekat dipermukaan gigi. Plak tersebut mengandung sumber bahan genetik bakteri yang berpotensi sebagai sumber informasi dari kesehatan manusia. Rangkaian genetik yang berupa DNA yang dihasilkan melalui serangkaian Ekstraksi DNA dan melalui penyusunan pada sampel kalkulus gigi. Penelitian ini dilakukan untuk mengetahui hasil identifikasi DNA bakteri apa saja yang ditemukan dari kalkulus gigi rangka manusia prasejarah. Penelitian ini dilakukan dengan metode studi pustaka melalui pendekatan sistematis dengan mengumpulkan artikel melalui mesin pencarian elektronik yang sesuai dengan kategori inklusi dan eksklusi. Dari 8 artikel yang memenuhi kriteria, 6 artikel berbentuk penelitian dan 2 artikel berbentuk review. Empat artikel meneliti penyakit pada manusia zaman purba hasil dari ekstraksi kalkulus gigi pada zaman paleolitik sampai neolitik awal, dua artikel meneliti dengan membandingkan sampel manusia modern dan manusia prasejarah dan dua artikel yang lain berupa review dari beberapa penelitian. Penelitian yang menggunakan analisis dari kalkulus gigi ini sangat berpotensi untuk menghasilkan beberapa informasi kehidupan pada Pra sejarah selain itu penelitian ini juga menguntungkan dalam penelitian di masa yang akan datang sehingga dapat dikatakan bahwa penelitian analisis DNA menggunakan kalkulus gigi ini sebagai *time capsule*, karena kalkulus gigi menyimpan banyak materi genetik yang berpotensi menyimpan banyak informasi penting.

Kata kunci: Manusia purba, DNA bakteri, kalkulus gigi

Abstract

Dental calculus is defined as mineralized dental plaque that is formed through the complex interaction between saliva and bacteria which adhere to the surface of the teeth. The plaque contains sources of bacterial genetic material which has the potential to be a source of information on ancient human health. Genetic sequences in the form of DNA are generated through a series of DNA extraction and through preparation of calculus samples of prehistoric human teeth. This research was conducted to determine the result of the identification of bacterial DNA found from ancient human skeletal dental calculus. This research was conducted using a literature study method through a systematic approach by

collecting articles through an electronic search engine that fits the inclusion and exclusion categories. Of the 8 articles that met the criteria, 6 were research articles and 2 were reviewed articles. Four articles examined disease in ancient humans from the extraction of dental calculus in the paleolithic to early neolithic periods, two articles examined samples comparing modern humans and prehistoric humans and two others were reviewed article. Research that uses analysis of dental calculus has the potential to produce some information on life in pre-historic times besides that this research is also beneficial in future research so that it can be said that DNA analysis research uses dental calculus as a time capsule, because in dental calculus store a lot of genetic material that has the potential to store a lot of important information.

Keywords: *ancient man; DNA bacteria; dental calculus*

INTRODUCTION

Ancient Human who lived long before writing era was discovered inhabited the earth about 4 million years ago. Based on several studies carried out by experts, it can be reconstructed several types of ancient humans that had lived in pre-literate times, namely Meganthropus, Pithecanthropus, and Homo Sapiens (Bani 2011).

Dental forensic science that specializes in oral and maxillofacial structures, this science help archaeologists to identify a discovery using the teeth of an ancient human skeleton that had been buried for millions of years even though its condition is incomplete and does not leave soft tissue remnants. Thus, skulls and teeth are used as alternative materials for identification, especially using ancient human dental calculus as the object of analysis (Rizqullah et al. 2017). Tooth can be identified in ancient humans since its ability to resist the decaying process. The decaying process will occur if there are four mutually continuous factors, namely the presence of the *host*, substrate, bacteria, and time. In prehistoric humans, the four factors of decay above are no longer working. The teeth also have heat resistance up to 1093℃ (Sudrajat, n.d.).

Dental calculus has been recognized as an informative tool for understanding dietary and health patterns in prehistoric times (Weyrich, Dobney, and Cooper 2015). Dental calculus is the calcification of dental plaque and various molecules in the oral cavity (McLeod 1996). Clinically looks like cement, has good adhesive *strength* and hardness to pressure on the Vickers scale of 30-40 U, with a maximum value of 190 U (Metcalf, Ursell, and Knight 2014). This material adheres to the natural surface of teeth or prostheses, based on topography with marginal gingiva as a limit, dental calculus can accumulate in the supragingival and subgingival (McLeod 1996). When the food is masticated in the oral cavity, plaque is formed by absorption of proteins and bacteria, and dental calculus is subsequently formed through the complex interaction between saliva and bacteria on the dental surface (Radini et al. 2017).

The etiology of dental calculus is multifactorial. The combination of diet, genetic factors, oral acidity, and oral hygiene, all of these factors lead to the accumulation of microorganisms and food debris itself which eventually calcifies into plaque through the process of mineralization (Eisenhofer et al. 2017). Dental calculus was then taken as a sample for examination of deoxyribonucleic acid (DNA). This DNA retrieval requires the

preservation of dental calculus in order to keep the microbes contained intact, then followed by an extraction technique and followed by sequencing of the bacterial DNA structure (Eisenhofer et al. 2017).

Deoxyribonucleic acid or also known as DNA is a molecule that stores some of the genetic information used in the development, function, and reproduction of all living organisms (Fagern€ et al. 2020). DNA from dental calculus was obtained through an extraction technique process that applied High Throughput Sequencing (HTS) and the metagenomic method (Fuente, Flores, and Moraga 2012). The information obtained after going through this process reveals information about the long-term impact of diet on health, disease, and the presence periodontal pathogens of certain bacteria over time. In connection with the above explanation can provide information about the potential of DNA using dental calculus analysis in the field of dental forensics.

METHOD

In this study, used the Systematic Review method using the method Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA). The research was carried out from July to December 2020 by searching for articles on the predetermined database, namely PubMed, Science Direct, and EBSCOHost.

Literature Search

The research was carried out using keywords or MESH Terms, namely Ancient Man, Dental Calculus, and DNA Bacteria. These keywords are then used together with Boolean operators (OR, AND) to combine searches.

Inclusion and Exclusion criteria

The inclusion criteria in this study were full-text articles, articles published in 2010-2020, and articles discussing the relationship between dental calculus and bacterial DNA in prehistoric humans. The exclusion criteria in this study were articles that did not pass the voting Rayyan QCRI and articles that discussed dental calculus other than human bacterial DNA.

Article Selection

Article selection by applying inclusion and exclusion criteria. After all relevant articles were collected and duplicate removed, the titles and abstracts of all remaining articles were screened to eliminate articles that did not meet the inclusion criteria. Then the full text of all the remaining articles is read and entered into Rayyan QCRI application to be selected based on the suitability of the content with the theme used for research by researchers and supervisors. In systematic writing, used a diagram as a part of the process of writing a systematic review because it allows the reader to see the researcher's process of obtaining all the references used in the study. The PRISMA diagram of this study is as follows:

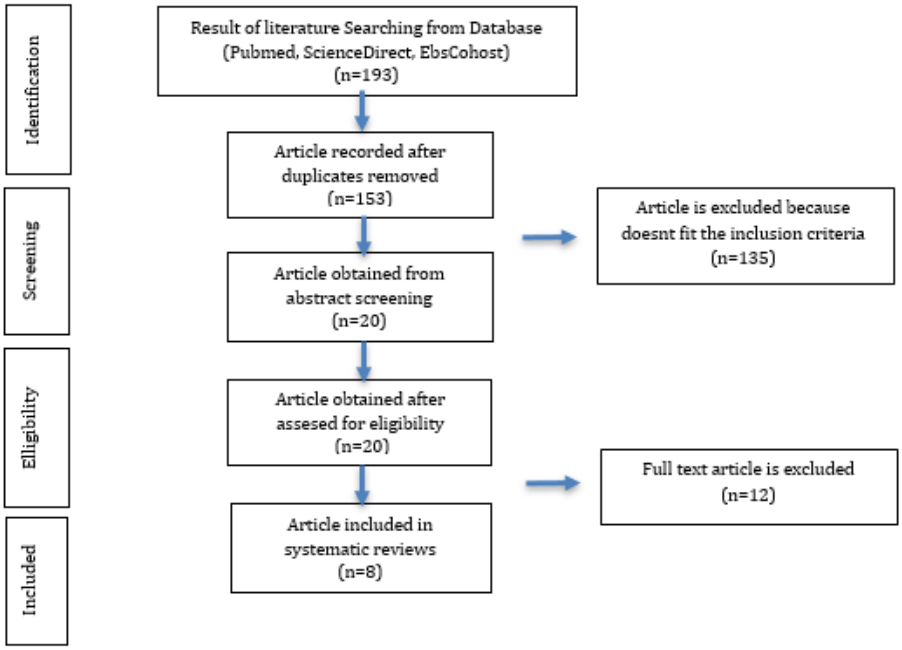

Figure 1. PRISMA *Flow Figure of Literature Search and Review*

Selected Journals Quality

Assessment or *critical appraisal* is a systematic process of evaluating and interpreting research results to consider the validity, results, and relevance of research (MacDonald 2014). The quality assessment used in this study *is the NIH Quality Assessment Tools. NIH Quality Assessment Tools* is a quality assessment tool in the form of a *checklist* that can be used for various types of studies and included the studies contained in this research.

RESULT

Study Description

The total number of articles that have been selected and reviewed are 8 articles which are identified based on the source of the article, year of publication, country of origin of the order, and the form of the article. The following are the result of the search that meet the criteria:

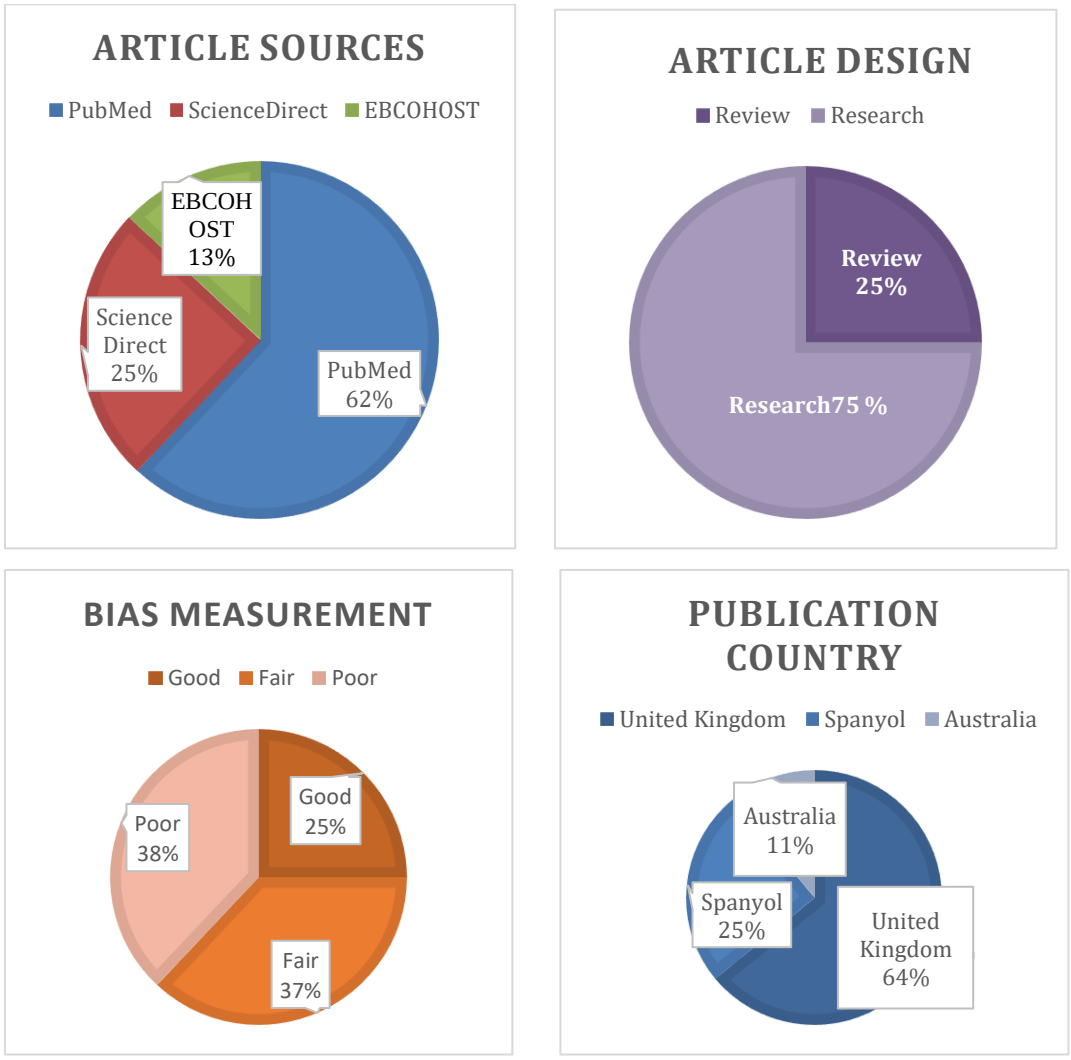

Figure 2. Distribution of articles by source, form, bias assessment and country of publication of articles

Based on Figure 2. These articles obtained based on criteria from several electronic search engines are as follows: five articles from *Pubmed*. Two articles from *Science Direct*. One article from *EBSCOHost*. It can be concluded that the search for the required keywords mostly comes from the electronic search engine *Pubmed*. In this study, the 8 selected articles were divided into 2 categories, namely articles in the form of research and in the form of reviews. From table 2. the presentation of the results of the articles that have been analysed there are 8 selected articles, and 2 articles have *good* quality (Weyrich et al. 2017; Velsko et al. 2017). Three other articles belong to the fair category (Weyrich, Dobney, and Cooper 2015; Fagernäs et al. 2020; Huynh et al. 2016). Three more articles have *Poor* quality or have a high potential bias (Velsko et al. 2015; Eisenhofer et al. 2017). In this study, most of the results for the publication of articles on dental calculus extraction were found in Australia.

Year of Publication

The articles used must also comply with the inclusion criteria in which the article was published maximum in the last 10 years, namely between 2010-2020.

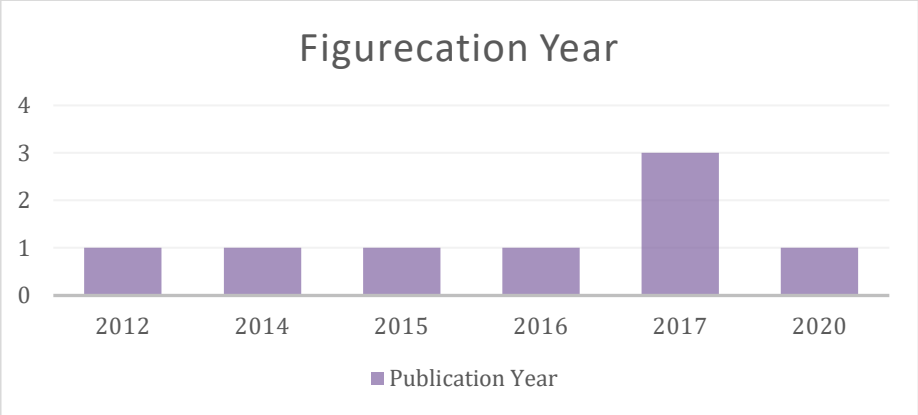

Figure 3. Year of Article Publication

Out of 8 articles obtained, 3 articles published in 2017, 1 article published in 2015, 1 article published in 2012 from search results *PubMed*, 1 article published in 2020, 1 article published in 2016, and 1 article published in 2014 from search results *Science Direct*, and 1 article published in 2020 from search results *EBSCOhost*. Based on the diagram above, it can be concluded that the articles obtained and according to keywords were most widely published in 2017.

Cultural Age

From the results of 8 articles reviewed by researchers, the following is a *timeline* of ancient human skeletons from various cultural ages based on table 2. Presentation of article results:

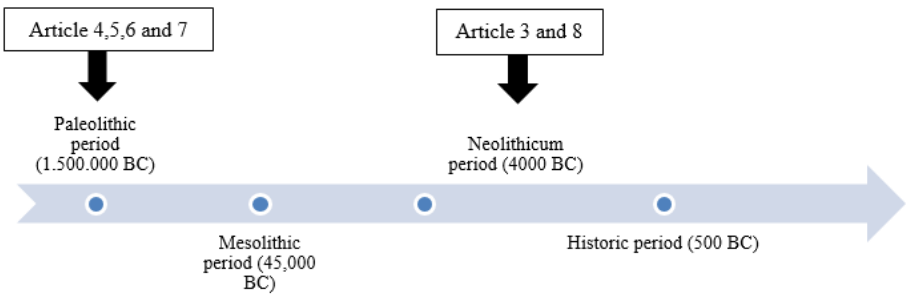

Figure 4. *Timeline of the* Culture Age of the Skeletal Sample

The oldest skeleton studied by Weyrich, *et al.* (2017) which has a skeletal age of about 40,000 years, namely in the Paleolithic era, while the skeletal sample used by Radini, A., (2017), Hardy, K. *et al.*, (2012), and Velsko, IM *et al.*, (2017) use samples

that have a cultural age in the Paleolithic era, and finally Huynh, HTT, (2016) and Adler, CJ *et al.*,(2013) examine samples that have a cultural age in the Neolithic era.

Table 1. DNA information of bacteria and micro-organisms found

Microorganisms Discovered	DNA information of bacteria and other microorganisms from found dental calculus	Source articles
Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, and *Spirochaetes*	Discovery of DNA of the same bacteria in modern and ancient humans from different period	Articles no 3, 5, and 8 in table 2. presentation of the results of the article
Enterocytozoon bieneusi (fungus)	Discovery of the fungal phylum that causes acute diarrhea	Article no 6 in table 2. presentation of the results of the article
P. gingivalis	Discovery of bacteria period-pathogenic as an indication of Periodontal Disease	Article no 6 in table 2. presentation of the results of the article
Penicillium (fungus)	The discovery of self-*medicating* aspirin to treat tooth abscess	Article no 6 in table 2. presentation of the results of the article
P. *gingivalis, Tannerella and Treponema*	discovery of Periodontal bacteria as an indication of systemic disease	Articles 3, 4, 7, and 8 in table 2. presentation of the results of the article
Clostridiales incertae sedis and *Veillonellaceae*	Discovery of bacterial DNA related to food spoilage	Article no 6 in table 2. presentation of the results of the article

Based on the 8 articles studied, information on bacterial and fungal DNA was found. The three articles studied have similar results which state that some dental calculus samples from bacterial DNA extraction can identify several diseases suffered in ancient times such as acute diarrhea, dental abscesses, and periodontal disease. Three other articles resulted in the discovery of fungi as antibiotics, the DNA of bacteria common to modern humans of various ages, and the discovery of bacterial DNA related to food spoilage.

Presentation of Results

Each article yielded information on potential bacterial DNA from different dental calculus. Hardy, K. et al., (2012) and Huynh, HTT, (2016) reviewed information by comparing bacterial DNA with dental calculus samples in prehistoric and modern human skeletons. While Weyrich, LS et al. (2017), Velsko, IM et al., (2017), and Adler, CJ et al., (2013) reviewed the relationship of prehistoric human dental calculus from bacterial DNA using calculus samples from ancient human skeletons with specific skeletal

samples. Weyrich, LS, Dobney, K. & Cooper (2015) and Fagernäs, Z. et al., (2020) produced a theory of the process of extracting bacterial DNA and techniques for compiling bacterial DNA from prehistoric human skeletal samples.

Table 2. Presentation of Article

No	Author	Article Title	Skeleton Sample	Skeleton Origin Country	Cultural Age	Strengths	Weaknesses	Bias Assessment
1.	Weyrich, LS, Dobney, K. & Cooper (2015)	*Ancient DNA analysis of dental calculus. Journal of Human Evolution.*	Early Neandert hal Human Skeleton	Europe, Australia	Not mentioned in the article	Research in the form of theory	Research in the form of theory	*Fair*
2.	Fagernäs, Z. *et al.,* (2020)	*A unified protocol for the simultaneous extraction of DNA and proteins from archaeological dental calculus. Journal of Archaeological Science, 118,*	6 human skeletons from time to time	1 individual each from Driffield Terrace, Wighill and Rupert's Valley and 3 individuals from San Martín de Dulantzi. (United Kingdom)	Not mentioned in the article	Research in the form of theory	Research in the form of theory	*Fair*
3.	Huynh, HTT, Verneau, J., Levasseur, A., Drancourt, M. & Aboudhara m, G. (2016)	*Bacteria and archaea paleomicrobiology of the dental calculus: a review. In Molecular Oral Microbiology (Vol. 31, Issue 3, pp. 234–242). Blackwell Publishing Ltd*	*4 ancient human skeletons*	*4 ancient human* skeletons originating from Dalheim, Germany	In the early neolithic era at vulnerable (10,000-12,000 years ago)	Dental calculus samples use samples that compare 2 periods, namely modern and medieval times which results in a comparison of the bacteria produced in diverse and clear, and detailed	The skeletal samples used are not detailed and the article does not mention the gender	Fair

4	Radini A., Nikita, E., Buckley, S., Copeland, L. & Hardy, K. (2017)	*Beyond food: The multiple pathways for inclusion of materials into ancient dental calculus. American Journal of Physical Anthropology.*	Humans In the Bronze Age	United Kingdom	In the Paleolithic Age (300,000-400,000 BC)	The DNA extraction process is described in more detail than the results of other studies.	No detail mentions the bacteria found	*Poor*
5.	Hardy, K. et al., (2012)	*Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus.*	13 Early Neandert hal Human Skeletons	*Neanderthal s (Western Europe)*	Early in the Paleolithic era (24,000-30,000 years ago.)	His research yielded information on drug discovery from DNA extraction	No detail mentions the bacteria found	*Poor*
6.	Weyrich, LS et al., (2017)	*Neanderthal behavior, diet, and disease were inferred from ancient DNA in dental calculus. Nature, 544(7650), 357–361.*	5 Skeletal samples from dental calculus Neandert hal Man	Neanderthal s (Western Europe)	Paleolithic vulnerable Era (40,000 years ago)	Dental calculus samples and the method used were explained in detail one by one so that the results of the bacteria were detailed to the point where information about the diseases suffered at that time	The skeletal samples used were not identified by gender	*Good*
7.	Velsko, IM *et al.,* (2017)	*The dental calculus metabolome in modern and historic samples. Metabolomics, I, 134.*	17 Dental calculus samples from ancient human	United Kingdom	Paleoltihic Era (17.000-20.000 years ago)	The extraction process is described in detail so as to obtain the results Bacterial DNA that obtained information on periodontal disease suffered at that time	The skeletal sample used was not detailed there is even a skeleton whose sex is not identified	*Good*

8.	Adler, CJ et al., (2013)	*Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nature Genetics, 45(4), 450–455*	34 Ancient Human Skeletons	Central Europe (Poland)	In the Early Neolithic era (10,000-12,000 years ago)	The dental calculus sample and the resulting process were described in detail so that it could produce bacterial DNA from the disease at that time	The skeletal sample used was not detailed there is even a skeleton whose sex is not identified	*Fair*

DISCUSSION

A study by Jessica L. Metcalf (2014) stated that dental calculus from ancient humans has the potential to produce profitable analysis in future DNA analysis research. Dental calculus can act as a time capsule that stores a lot of genetic material that has the potential to store a lot of important information for DNA analysis. This strategy of using DNA from dental calculus is referred to as a source of information targeting DNA on pathogens responsible for diseases such as plague and cholera (Velsko et al. 2015).

The results of research by Weyrich (2014) and Adler (2017) produced the same results, namely the discovery of 6 oral bacteria that dominate the dental calculus in this ancient human skeleton such as bacteria in the phylum Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria, Proteobacteria, and Spirochaetes that already known predominate in humans today, which were sequenced using High Throughput Sequencing (HTS) which were sequenced based on the Human Oral Microbiome Database (HOMD).

The study by Adler, CJ (2014) who examined 34 ancient human skeletons (11 males, 11 females, and 12 unknown sexes) at the age of 20-60 years of death from the mesolithic-medieval era showed dental calculus in samples examined by comparing samples of ancient humans and samples of modern humans resulted that ancient human calculus in this neolithic era was dominated by bacteria from the phylum Firmicutes as much as 33% and in modern human samples contained bacteria with the same phylum as much as 50%. This study also showed that in ancient human samples there were also 15 common bacterial phyla that were present in modern humans 19% of it is the phylum actinobacteria (Adler et al. 2013).

The research from Laura S. Weyrich (2017) from 5 samples of ancient Neanderthal human skeletons showed that the dental calculus in these skeletal samples had been contaminated by the environment, as evidence in samples of El Sidron 1, El Sidron 2, and spy II whose DNA composition is approximately 93.76% bacterial, 5.91% archaic, 0.27% eukaryotic and 0.06% viral identifiable sequences. There are approximately 222 species of bacteria in each individual studied. In El Sidron 1 samples, DNA was also found perio-pathogenic bacteria indicating periodontal disease from there the researchers also discovered self-medicating practice by using natural pain killers

namely salicylic acid which belongs to aspirin class drugs. There were also found the usage of fungi, *Penicillium*, which contain natural antibiotics. In addition to bacterial DNA in this sample, other microorganisms were also found, namely the intracellular pathogen eukaryotic microsporidia or called (*Enterocytozoon bieneusi*), which is one of the fungi that cause acute diarrhea (Weyrich et al. 2017).

The results of research by Adler, CJ (2014) who examined 34 ancient human skeletons (11 male, 11female, and 12 unknown sex) samples including individuals from the last hunter-gatherer era in Poland, individuals at the beginning of agriculture in Central Europe, as well as the late Neolithic, and then the rural and urban Bronze Age in the Middle Ages. Using PCR Techniques and DNA Sequencing techniques proved that early Neolithic human samples with hunter-gatherer groups showed less association between caries-related disease and periodontal disease as evidenced by a decrease in the amount of carbohydrate food compared to the hunter-gatherer era, the evidence for this decrease was influential. on the periodontal disease during the transition from hunting to farming. Farmer groups from the Neolithic era showed more periodontal disease as evidenced by the discovery of taxa from the DNA of the bacteria *Clostridiales incertae sedis* and *Veillonellaceae* which are associated with decay bacteria. Ancient human populations in the early neolithic period show more periodontal diseases, including *P.gingivalis, Tannerella,* and *Treponema*.

Research from Velsko (2017) in line with research by Adler, CJ (2014), from 17 samples studied using UPCL-MS/ found that several amino acids were grouped into 8 groups of amino acids namely Carbohydrates, Cofactors and vitamins, Energy, Lipids, Nucleotides, Peptides, and Xenobiotics and in one of the samples found gram-positive bacteria in ancient humans including *actinomyces* that have formed spores and associated with periodontal disease.

CONCLUSION

Based on the results of the study, it can be concluded that with the dental calculus analysis research using bacterial DNA is very useful for archaeologists and odontologists to know the history of the past and future are like a time capsule, because dental calculus had a lot of genetic material and potential to keep a lot of important information. In this study, we found out that Bacterial DNA in dental calculus using this extraction process can also potentially yield some information from microorganisms present in samples of ancient human calculus, such as the discovery of bacterial DNA and fungi that cause acute diarrhoea, periodontal disease, and several groups of fungi are also found which was used as an antibiotic in ancient times.

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