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Sex Determination (X and Y Chromosome) based on Histological Findings in Tooth: Literature Review

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Abstract

Odontology forensic is a branch of forensics science as well as dentistry that handles, examines, and evaluates dental evidence in effort of complying the interest of justice. The ability of dental evidence to be used as an identification tool is very possible as the fact of how durable teeth are. There are several methods to examine the dental evidence in effort to conduct an identification. However, there are circumstances in which histological examination is the only method that can be conducted. This led to the aim of this study. To review histological examination in teeth as sex determination method and to discuss its exclusivity compared to other methods. This literature used journals and textbooks with keywords according to the topic of discussion. Data obtained from different search engines such as Google Scholar, PubMed, Springer and NCBI. A total of 27 journals and 3 textbooks skimmed and narrowed down in to the 12 journals and 1 textbook. Narrowing down the data are necessary as information attained are similar to avoid redundancy. There are two findings that are targeted when it comes to histological examination of the tooth in order to determine one's sex. They are F bodies and Barr bodies. Both targets appear in sample obtained from the dental pulpal tissue. Further, histological examination as a sex determination method is critical to be develop. Especially in times whereas the other methods are not reliable enough due to limited sample inclusion and cost. It is important to understand histological examination in forensic dentistry.

Keywords
Sex determination; tooth histology; Barr bodies; F Bodies; X Y Chromosome

I. Introduction

Odontology forensic is a branch of forensics science as well as dentistry that handles, examines, and evaluates dental evidence in effort of complying the interest of justice. This includes the usage of dental evidence to provide information to the authority (Divakar, 2017). The ability of dental evidence to be used as an identification tool is very possible as the fact of how durable teeth are. It is proven by its perseverance even after the death of the individual and further that can endure 1600 degree Celsius (Carbot, 2013 in Divakar, 2017). With the advancement of technology, the information provided by dental evidence has gone beyond dental identifications which includes things such as but not limited to age assessment, bitemark analysis, even further recognition and reporting of child abuse (Schrader, 2018 in David & Lewis, 2018). Dental evidence includes unique characteristics of individuals' antemortem and post-mortem dental condition. It can be presented by the remains' set of teeth, recorded dental charts, radiographs, and or other types of dental evidences (Krishan, Kanchan & Garg, 2015). Those types of dental evidences can further

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establish the remains' identity because dental evidence can determine one's race, age, and sex. There are several methods to examine the dental evidence in effort to conduct an identification. Specifically in sex determination, there are four methods which includes the usage of morphological characteristic, morphometric characteristic, histological examination, and DNA analysis of the teeth (Syafitri, Auerkari & Suhartono, 2013). Morphological characteristic and morphometric characteristic are limitedly can be done if the dental evidence is found in a full form manner. On top of that, DNA analysis requires advance technologies and higher cost to be conducted. Therefore, this paper aims to review histological examination in teeth as sex determination method and to discuss its exclusivity compared to other methods.

II. Research Method

The method used is a literature review from references that are in accordance with the topic of discussion. This literature used journals and textbooks with keywords according to the topic of discussion. Data obtained from different search engines such as Google Scholar, PubMed, Springer and NCBI. A total of 27 journals and 3 textbooks skimmed and narrowed down in to the 12 journals and 1 textbook. Narrowing down the data are necessary as information attained are similar to avoid redundancy. Literature Review Sex determination can be done by histological examination. The examination will need dental evidence to do so. Dental evidence that can be used for sex determination based on histological findings are such as but not limited to buccal smears, blood, bone, skin biopsy, hair root sheath and tooth pulp (Rai, 2010 in Rai & Kaur, 2013). As this paper will specifically limit the discussion on histological findings in teeth and only for the aim of sex determination, it is important to understand which part of the teeth can be used to do so, how to obtained it and what to see under the microscope. Veeraragravan, et al. (2010) along with other study from Khorate, et al. (2014) both used dental pulp as their dental evidence in conducting research about sex determination using histological findings. In order to obtain ideal sample of dental pulp there are steps as such as.

2.1 Creating section of the teeth

In creating section of the teeth there are different types of drill or bur are used. Veeragravan, et al. (2010) used carborundum disc at 3000 rpm to separate the crown while Khorate, et al. (2014) used cylindrical diamond bur. Further, after drilling from the lingual surface of the teeth, the usage of mallet and chisel is needed in opening up the teeth to access the pulpal tissue.

2.2 Curetting the pulpal tissue

In curetting the pulpal tissue from the cavity, Khorate, et al. (2014) utilized a probe also a #10 broach.

2.3 Washing the pulpal tissue

Washing the pulpal tissue before processing it further is important in order to washed out any debris and calcified particles. Normal saline is used to wash the pulpal tissue then fixative in form of 3 Methanol: 1 Glacial acetic acid is used to keep the pulpal tissue (Khorate, et al., 2014).

2.4 Processing the pulpal tissue

In effort to soften the pulpal tissue, both Veeraragravan, et al. (2010) and Khorate, et al. (2014) acetic acid added into the pulpal tissue. 20% Acetic acid added to the pulpal tissue in the mortar. Then the suspension centrifuged for 5 minutes with REMI RM12C micro centrifuge at 6000 rpm. A monolayer of the centrifuged suspension on chilled microscopes slides obtained and ready to be placed into the fluorescence microscope. This mono-layering technique is done in order to get a homogenous population of cells. To continue, those samples were air-dried and fixed with absolute methanol (Khorate, et al., 2014).

2.5 Staining the sample

The addition of certain stains is done based on what is aimed to be seen under the microscope (Khorate, et al., 2014).

III. Result and Discussion

It led this paper to further review what are exactly the options to see with those samples under the microscope in the context of sex determination. Firstly, the presence or absence of the X-Chromatin and intranuclear structure. This has become one prominent way to determine sex. The X-Chromatin and intranuclear structure itself often called as Barr body (Rai & Kaur, 2013). Historically, the discovery of Barr bodies began while Barr and the other observers were observing a cat. They found unique cell that particularly existed in female mammalians and specifically in their nuclei somatic cell (Rapport, 2014). It is also has been found that Barr bodies are stable even when it is exposed in such high temperature (Suazo, et al., 2011). This indicate that the stability of Barr bodies which can help the identification in mass disaster that involves with heat such as bombings, volcano eruption, and so forth (Khanna, 2015). Although, the study of heat exposure was conducted in a in vivo manner which sparks debates upon the idea of whether the environment in real-life settings is similar to the in vivo environment. There is an argument saying that in the real-life settings, there are a lot of other layers that protects the teeth such as the oral cavity. The argument then concludes that, comparatively, the in vivo heat exposure is not the same with the real-life settings (Kalistu & Doggalli, 2016).

Figure 1. *Barr body (Rai & Kaur, 2013).*

Barr bodies usually located in the edge of the nucleus although it also rarely can be found in the nucleus (Syafitri, Auerkari & Suhartono, 2013). Barr bodies are mostly sized about 0.8 x 1.1 microns and are basophilic structures. Barr bodies appear in different shapes. At times they are seen as spherical, rectangular, biconvex, triangular and or plano-convex (Ramakrishnan et al., 2015). In investigating the presence of Barr bodies, the stain

that often used is Hematoxylin and Eosin. It is proven as in Khorate, et al. (2014), Rapport, et al. (2014), and Veeragraraghavan, et al., (2010) all use Hematoxylin and Eosin stain to see the presence of Barr Bodies. It is enough to use ordinary microscope to investigate the presence or absence of the Barr Bodies.

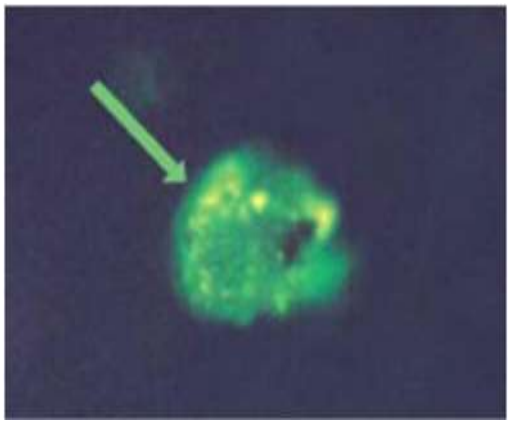

Figure 2. *F-body seen in Y-Chromosome of Dental Pulp Tissue (Syafitri, Auerkari, & Suhartono, 2013).*

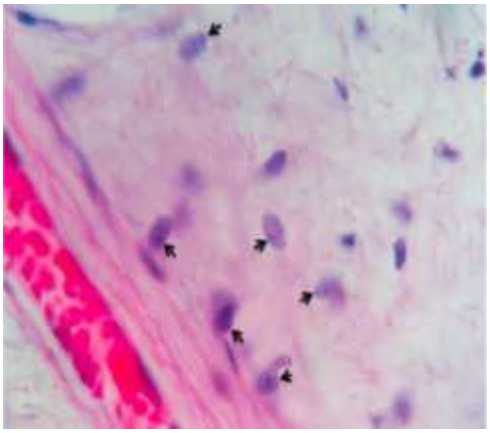

Figure 3. *Dental pulp tissue with visible Barr bodies stained by haematoxylin eosin (Suazo, 2010 in Rapport, 2014).*

Secondly, other than Barr bodies, it is also well known that the presence of F bodies of the YChromosomes can also be the determinants of sex in odontology forensic. Under ultraviolet light, the fluorescent dye binds strongly to the Y- Chromosomes then fluorescent spots will be visible. Those spots are considered as F bodies (Kalistu & Doggalli, 2016). The stain used in identifying the F bodies is quinacrine dihydrochloride which also well-known as quinacrine mustard (Syafitri, Auerkari & Suhartono, 2013). Three different studies done by Veegraraghavan, et al. (2010), Rapport (2014), and Khorate (2014) all used quinacrine dihydrochloride to do so. Samples will then be placed in the fluorescent microscope to see whether the F bodies are present or absent.

The presence of F bodies confirmed by the existence of fluorescent spots that appear in the Y- Chromosomes. Contrary to that, if there is no appearance of the F Bodies, the cell considered as a female cell. However, this technique of fluorescent staining of the Y-Chromosomes can only be done limitedly on healthy pulps. Teeth with caries that involve with its dentin are excluded from this technique (Ramakrishnan et al., 2015).

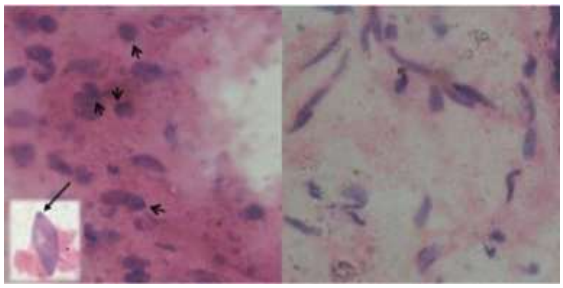

Figure 4. *Comparative view between the presence of Barr Bodies indicating female cells (left) and the male cells proven by the absence of Barr bodies (right) in dental pulp tissue with Haematoxylin and Eosin stain (Khorate, et al., 2014).*

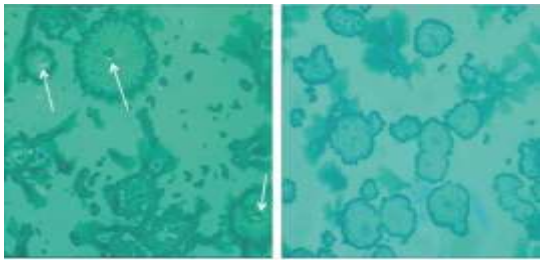

Figure 5. *Comparative view between the presence of F Bodies indicating male cells (left) and the female cells proven by the absence of F bodies (right) in dental pulp tissue with Quinacrine dihydrochloride stain (Khorate, et al., 2014).*

Discussion

In order to be able to compare histological examination as a method of sex determination we need to further understand the challenges in this technique. Based on different literatures, there are different factors have been identified as the challenges.

a. Time sensitivity

Different studies have proven that, time interval between the tooth extraction and the histological examination influence the accuracy of the determination. The study conducted by Veeraraghavan, et al. (2010) divided the teeth samples into three groups. One group is a group of teeth which are examined immediately after the extraction, the next one is a group [of teeth which are examined one month after the extraction, and the last group is the group that is examined five months post extraction. In the immediate examination and onemonth prior tooth extraction groups, the rate is 100% correct in determining the Y chromosome. However, the accuracy shown to be decreasing in the 5 months prior to tooth extraction examination. On the other hand, in case of the females' tooth the accuracy is higher in the immediate examination group compared to the one month and five months prior the extraction examination group. Similar findings are found in another study conducted by Khorate, et al. (2014). In that study, the number of Barr bodies and F bodies gradually decreased as with the longer time interval between the extraction and examination takes. This to say, the accuracy of the sex determination method dropped as the longer the time interval is.

b. Advancement in staining procedure

Staining procedure is a sensitive procedure as it is very contingent to the technique. It is important to get a homogenous monolayer of cells from the suspension on the slide because it will prevent the debris or another cell to mask the fluorescent F Bodies (Khorate, 2014).

c. The quality of the dental pulpal tissue

There have been studies investigating the potential factors that potentially disturb the quality of the dental pulpal tissue in order for it to be decent enough as samples. Decent enough means that the dental pulpal tissue still able to present histological view that can determine sex of the remains. Study about how high temperature exposure can intervene the dental pulpal tissue quality has been done. The study done by Suazo, et al. (2011) showed that the dental pulpal tissue quality is still decent up until 400 degrees Celsius of heat exposure. However, in that study as well, at 600 degrees Celsius, the histological view of the tissue appeared disorganized which makes it hard to identify the presence of the Barr bodies. Further, at 800 and 1000 degrees Celsius it is impossible to do any tissue analysis. Therefore, high temperature up until 400 degrees Celsius does not intervene the pulpal tissue quality. To continue, other factor to be considered is the humidity of the environment. The humidity of the environment has been proven as one of the challenges in doing histological examination of the dental pulp tissue. As the humidity arise, the process of decomposition by bacteria degrades the clear visibility of the chromatin observation which leads to inability to detect the presence of Barr bodies (Das, et al., 2004 ; Khorate, 2014).

Not only Humidity also proven reduce the amount of detectable F bodies as autolysis is accelerated by humid and wet environment (Dogalli & Kalistu, 2016). Hence, humidity of the post extracted teeth should be considered if histological examination would like to be conducted. With those challenges listed above, it becomes important to review the other methods in order to be able to compare histological examination as a method of sex determination to other methods. Other than histological examination, there are morphological characteristic analysis, morphometric characteristic analysis, and DNA analysis (Syafitri, Auerkari & Suhartono, 2013).

First of all, there is morphological characteristic analysis. Morphological characteristic analysis tends to be unreliable as there are conflicting evidences of sizes (Veeraraghavan, et al., 2010). Then, there is morphometric analysis. 2D and 3D measurement has been developed in practicing the morphometric analysis. Not just that, morphometric also used discriminant function analysis (García-campos, et al., 2018). However due to different size and variations of metric elements, discriminant function can only be applied to the same population (Rapport, 2014). This is also supported by a study underwent by Garcíacampos, et al. (2018) whereas it portrays the importance of morphometric analysis to be tested on different population groups considering the fact that sexual dimorphism in teeth may varies in each population.

Lastly, the other method of sex determination is by conducting Deoxyribonucleic acid (DNA) analysis. DNA analysis has the highest accuracy therefore is very reliable. Polymerase chain reaction (PCR) and RT-PCR are popular methods that are being used to conduct the DNA analysis (Subramaniam & Rohit, 2018). Based on different studies there are different samples that can be used along with this technique. Not only the dental pulpal tissue, but also epithelial tissue that can be found intraorally. Other than that, epithelial tissues that are found on toothbrushes can also be used as DNA samples (Maulani & Auerkari, 2020).

However, DNA analysis costs high number of collateral due to the fact that it requires advance technologies in order to be conducted. DNA analysis to determine the remains' sex is not only by finding Y- STR but can also be done by analyzing the number amel gene present in the sample (Ramakrishnan et al., 2015). Within those explanations, this is where histological examination steps in. Based on the study conducted by

Veeraraghavan, et al. (2010), histological examination is reliable, cost effective and simple. However the accuracy is limited for immediate examination only.

Rapport (2014), histological examination is reliable enough regardless the time interval of the remains' death and the histological examination. However the study was not conducted using tooth pulp samples, instead it was conducted using cortical bones sample (in this case can be assessed with mandibular jaw) even if it is not an immediate case. Further research is needed as it raises the question of why are there less accuracy in the usage of tooth pulp sample compared to cortical bone sample result even if both of the examination not conducted immediately.

IV. Conclusion

There are two findings that are targeted when it comes to histological examination of the tooth in order to determine one's sex. They are F bodies and Barr bodies. Both targets appear in sample obtained from the dental pulpal tissue. Further, histological examination as a sex determination method is critical to be develop. Especially in times whereas the other methods are not reliable enough due to limited sample inclusion and cost. It is important to understand histological examination in forensic dentistry.

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