Short Communication

Sibling Indices as Comparisons in Personal Identification Process through Short Tandem Repeats [STR] Loci CSF1PO, THOI, TPOX, vWA of Maduranese Ethnic in Surabaya

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ABSTRACT
Sibling indices can be used as a comparison through alleles Short Tandem Repeats [STR] loci. This is an observational study among Maduranese with 4 STR loci (CSF1PO, THOI, TPOX, vWA) obtained from their blood samples. The percentage of alleles shared: 82.5% [33 times] with 2 allele sharing, 12.5% [5 times] with 1 allele sharing, and 5 % [2 times] with 0 sharing alleles. Sibling indices (SI) calculation results: 65% of sibling indices pairs have SI greater than 100 and 15% of them were between 10-100 (strong and very strong). Sibling indices interpretation is supported; therefore, the claimed sibling indices relationships were indeed true among Maduranese ethnic group in Surabaya.

Keywords: Allele sharing, Maduranese , sibling indices, STR DNA

The identification process through deoxyribonucleic acid [DNA] analysis is based on the allele comparison process, which is through a sample that is found and compared to another allele from another person in the family line, especially from the parents or children. The allele is known to use the Short Tandem Repeats [STR] loci is widespread through human genome and the largest source of polymorphic markers that can be acknowledged by Polymerase Chain Reaction. Determining STR is a strict process, the purpose of identification using STR is useful in various cases such as when determining a perpetrator of a violent act (murder and sexual assault) (Butler 2006; Butler & Hill 2012; Butler 2015).

In certain conditions, such as the absence of parents or children, comparison f rom a close family line is needed as one of the alternatives for forensic DNA analysis process, such as from sibling indices (Fung et al. 2004; Consentino et al. 2015; Abbas et al. 2018). Sibling indices that are involved in forensic identification process are also taken their alleles at STR loci which is located at nuclear DNA. However, the use of sibling indices as a comparison through STR is still unknown in Indonesia and other countries

despite demands from ethnic diversity, population, natural disasters, and many other events that require forensic preparedness (Consetino et al. 2015; Karbeyaz et al. 2016).

This identification process through STR loci is important for broader purposes such as determining sibling indices. Genomic variation occurs beyond geographic boundaries, individual loci, and population-based STR accuracy determination. Determination of inclusive STR in a country not only helps in database and reference point formations, but also a series of known core STRs is able to effectively determine a population (Untoro et al. 2009; Maeda et al. 2015; Abbas et al. 2018).

The reason behind the selection of Maduranese ethnicity in this study is that in general, Maduranese is generally known to be temperamental and has a distinctive accent. Most Maduranese people have high work ethic and adventurous spirit, which make them prefer to migrate from their place of origin, and in fact, 20-30% of Surabaya population is of Maduranese descent (Prastowo et al. 2018; Sosiawan et al. 2019).

This study aims to analyze allelic sharing of STR loci in sibling indices among the Maduranese ethnic group in Surabaya as an alternative source of forensic identification in the absence of their parents. This study also uses four kinds of STR loci (CSF1PO, THOI, TPOX, vWA) as the main STR loci. It is not the miniature of the previous study conducted by Sosiawan et al. (2019) that has been published because there are two things that strengthen this statement, such as: 1. The CODIS used in these two journals are different, 2. The populations used in these two journals are different. The previous journal talked about the Maduranese population lived in Madura Island. This journal talks about the Maduranese population lived in Surabaya, so the subject is very different. The uses of these four STR loci are based on the previous finding by Prastowo et al. (2018). His study mentioned that these four STR loci had the highest power discriminant in Maduranese, so it was suitable to use it as an alternative source of forensic identification in the absence of their parents (Prastowo et al. 2018).

Forensic identification means that identification must be done to prove someone's relationship (origin of the child, paternity cases, genealogical relation, or identifying unknown crime victims) therefore there should be no errors in its identification. One of the Forensic Identifications can be done using deoxyribonucleic acid (DNA) examination because it has achieved greater recognition in supporting Indonesia's law enforcement (Atmaja 2005; Karni et al. 2013; Yudianto & Setiawan 2020). This study also used Maduranese populations because these populations are close to the mobilization to Surabaya City.

The sample of the study was volunteers' DNA from families consisting of parents and children. This study has received ethical eligibility from the Dentistry Faculty of Airlangga University No: 275 / HRECC.FODM / VI / 2020. This research conducted by using a total of 40 blood samples taken from a total of 10 families. Each family consists of a biological father, mother, and two children who are not twins. All samples collected in blood collection tubes marked with the letters F [father], M [mother], and S [child] to represent samples from the biological father, mother, and child, and isolated DNA pellets in each pair contains 10 DNA pairs added with 50 μl of distilled water (Chomczynski et al. 1997).

DNA amplification process was carried out via PCR-STR process (PowerPlex® 21Systems, Promega, USA) which objected to characteristic DNA sequence area to make a number of the isolated DNA's copies. Multiplication process of all 40 samples used 4 autosomal STR locus (CSF1PO, TH01, TPOX, vWA) using primers CSF1PO: 5'-AACCTGAGTC TGCCAAGGACTAGC-3' and 5'-TTCCACACACCACTGGCCATCTTC-

3', THOI: 5'-CTGGGCACGTGAGGGCAGCGTCT-3' and 5'-TGCCGG AAGTCCATCCTCACAGTC-3', TPOX: 5'-ACTGGCACAGAACAGGC ATCTAGG-3' and 5'-GGAGGAACTGGGAACCACACAGGT-3', vWA: 5'-CCTAGTGGATGATAAGAATAATCAGTATG-3' and 5'-GGACAGA TGATAAATACATAGGATGGATGG-3'. The PCR settings loci CSF1PO, THOI, TPOX, and vWA were used as a manual procedure derived from Promega (Gene Amp^{r}PCR System 9700 Thermal Cycler, Promega Corp. 2001). After being amplified by PCR, the PCR results were electrophoresed vertically with polyacrylamide agarose gel [PAGE] 6% [Bio-Rad Mini-PROTEAN®] with Silver Nitrate staining.

Sample's Allele Profile through Allele Sharing The readings from electrophoresis gel were alleles for each locus with K562 as control. The analysis was based on allele sharing frequency from kinship analysis, where the sibling indices' STR loci were used to determine specific STR loci that have kinship among Maduranese in Surabaya. Furthermore, it is analyzed through allele sharing as shown in Figure 1. Furthermore, the Sibling indices Index/full sibling indices index/Sibship index (SI) was determined. SI is calculated based on kinship analysis in the equation (Slooten 2011). Allele probability is taken from the allele frequency of the Indonesian population (Untoro et al. 2009). Combined sibship indices (CSI) were determined by multiplying individual SI values of the selected locus and projected for each pair locus. (Wenk 1996) (Table 1).

Based on Figure 1, allele sharing percentage in the current study from 40 observations (10 pairs x 4 loci) in sibling indices were as followed: 82.5% [33 times] with 2 allele sharing, 12.5% [5 times] with 1 allele sharing and 5% [2 times] with 0 allele sharing.
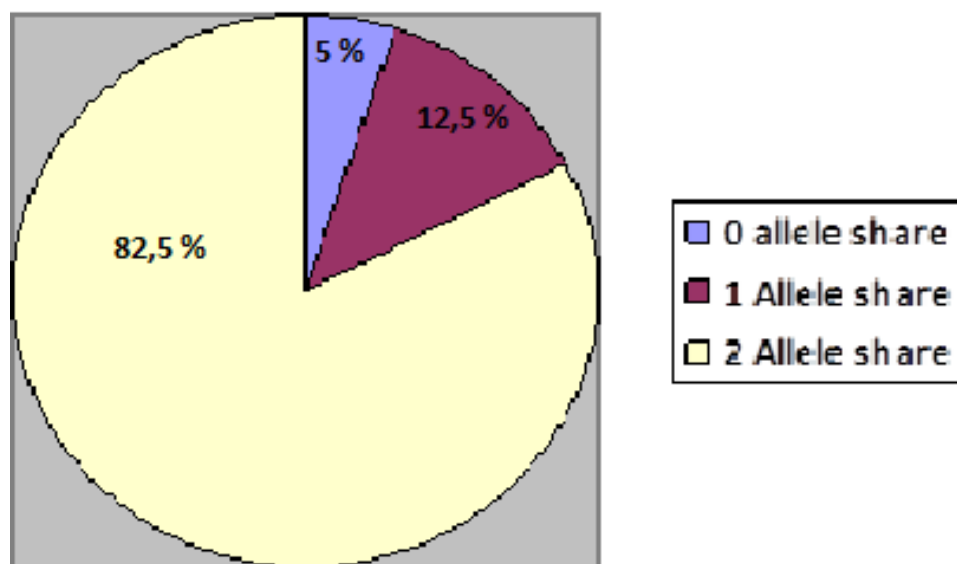

Figure 1. Allele sharing percentage in sibling indices.

Sibship Indices [SI] calculation in this study is shown in the Table below:

Table 1. Sibship Indices [SI] percentage in this study (Wenk 1996).

Probability ration in the form of *Sibship indices* (SI)	Strength	Percentage
<1	Weak	10%
1 – 10	Moderate	10%
10 -100	Strong	15%
>100	Very Strong	60%

Sibling Indices (SI) are shown in both table 1 and based on Figure 2 calculation, they showed that 65% of sibling pairs had SI greater than 100 and 15% of them had SI between 10-100. Its SI could be categorized as strong and very strong.
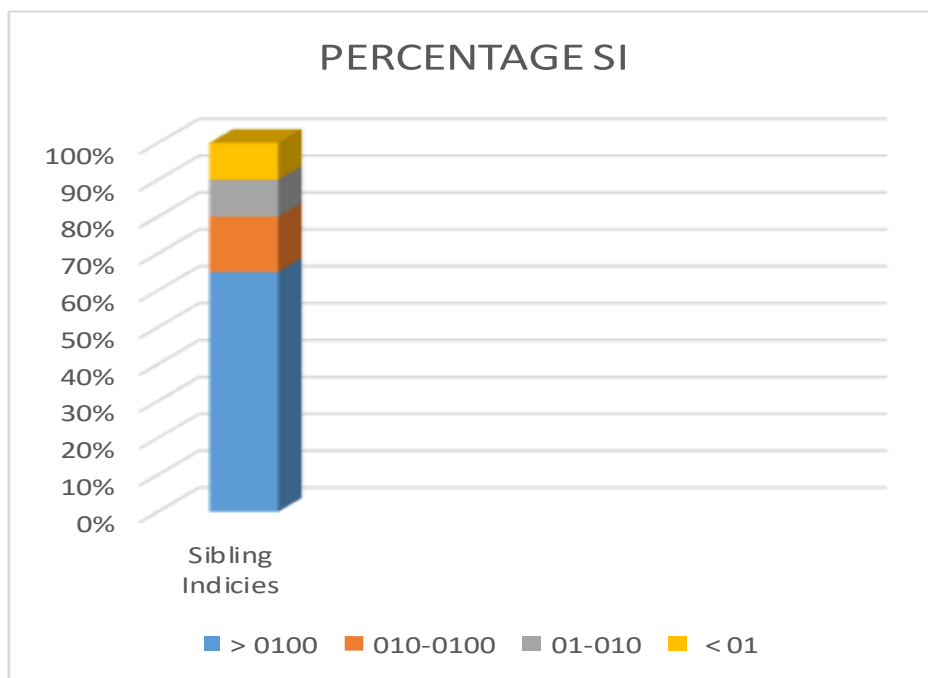
Figure 2. Percentage of Sibling Indicies (SI) in this study.

Areas containing repeating nucleotide sequences (eg: STR sequences) attract the attention of forensic experts because they contain a lot of information about variations and can be used for human identification. Repeating nucleotide sequences is used in identification, primarily in paternity tests. The paternity test is the use of a DNA profile to specify whether an individual is the biological parents of other individuals. A paternity test can be especially important when the rights and obligations of the father are at issue and the paternity of the child is in doubt. The test can also predict the probability of becoming a biological grandfather. There are older methods that also exist as a genetic testing to predict the probability of becoming a biological grandfather, such as ABO blood group, analysis of various proteins and enzymes (Human Leukocyte Antigen). (Omran et al. 2009; O'Connor 2011; Kido et al. 2003; Hares 2015; Marano et al. 2019).

STR has alleles for each locus. In allele examinations, allele frequencies are presented in the form of homozygosity, heterozygosity, the effective number of alleles (n), polymorphism information content (PIC), the power of discrimination (DP), and the power of exclusion (PE). A good index for the genetic polymorphism is based on the number of alleles because of the presence of characteristic alleles in the population. Therefore, it is necessary to carry out genetic screening before conducting a study. It is theorized that the larger the population size, the number of alleles observed will be also increased.

If the biological parents or children are not present in the paternity test, a comparison is needed from a close family line as an alternative for identification checks through DNA, such as from sibling indices. The use of sibling indices as a comparison is one of the identification methods (Reid et al. 2008; O'Connor 2011). Sibling indices will share zero or two identical alleles through offspring at certain loci with the same probability of 0.25 and share one allele with a probability of 0.5 (Wenk 1996; O'Connor 2011). In

the current study, sibling indices shared 5%, 12.5%, and 82.5% of zero, one, and two identical alleles, respectively, at CSF1PO, TH01, TPOX, and vWA loci. This is a different result from a study by Wenk (1996). This difference is likely caused by ethnicity that has different genetic contributions between populations. This can be attributed to historical and demographic processes that lead to genetic drift (Yamamoto et al. 2003).

In Sibling indices (SI) calculation, 65% of sibling indices pairs had SI greater than 100 and 15% of them had SI between 10-100 (strong and very strong), indicating that the use of STR loci CSF1PO, TH01, TPOX, and vWA might be very predictive to identify sibling indices from the Maduranese ethnicity in Surabaya.

If SI is below 10, then two alternative study options can be made. First, the study must use other loci that are still related to the selected locus. The second is that the study could use additional DNA testing from their uncles, aunts, and cousins. The first alternative is based on the Combined Sibling Indices (CIS) guideline for test results, where 90% of the test loci have a strong probability. The final alternative is in accordance with CIS Guidelines for evaluating sibling indices DNA testing (Wenk et al. 1996; Slooten et al. 2011).

This study indicates that two allele sharing is the strongest compared to one allele sharing and zero allele sharing. The value of 82.5% with two allele sharing indicated that the sibling indices's interpretation is fully supported, therefore the claimed relationship is true. Difficulties in the form of minimal DNA samples that were obtained in this study can be overcome by using other loci associated with the selected locus or additional DNA from uncles, aunts, and cousins.

AUTHORS CONTRIBUTION
A.Y. designed the research and supervised all of the processes, S.M.M.N. collected and analyzed the data, F.S. wrote and submit this manuscript.

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CONFLICT OF INTEREST
The authors declare that there are no conflict interests in publishing this article.

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