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Novel method to classify varicocele using electronic nose

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ABSTRACT

The prevalence of varicocele is estimated to be around 15-20% of the male population, and of these, about 35-40% are patients requiring infertility treatment. A varicocele is a dilation of the veins in the spermatic cord diagnosed by physical examination of the male genital area and assisted by scrotal ultrasound. The development of electronic nose technology provides an opportunity to detect disease characteristics of volatile organic compounds produced by biological materials. This study aims to utilize the metabolomic gas produced from the odor of the seminal fluid by using an electronic nose. The identification of the pattern of volatile organic compounds formed was labeled as unilateral varicocele, bilateral varicocele and clinical non varicocele as the basis for classification with supervised machine learning. In this study, the accuracy values were quite good for several algorithms, both in training accuracy and testing accuracy with an average accuracy value above 80%.

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1. INTRODUCTION

In the case of infertility worldwide, it is estimated that one in six couples is caused by male infertility. Usually related to sperm count, sperm vitality, sperm motility, sperm morphology, semen volume, and sperm deoxyribonucleic acid (DNA) integrity. Efforts to develop more accurate diagnostic methods are continuously being carried out because the clinical semen analysis method shows a sensitivity of about 89.6% with the ability to accurately diagnose 9 out of 10 infertile men. Routine examinations carried out were measuring sperm concentration, motility, and morphology as the main indicators and supported by other abnormal semen parameters. Several conventional methods for sperm analysis include using a counting chamber with hemocytometry methods, computer-assisted semen analysis, and spectrophotometry [1].

Varicocele is defined as dilatation or widening of the pampiniform venous plexus and causes retrograde flow due to incompetent venous valves. The prevalence of varicocele is estimated to be around 15-20% of the male population, and of this number, about 35-40% are patients who require infertility treatment. Varicocele itself is diagnosed by physical examination of the male genital area and along with the development of ultrasonography, cases of varicocele are easier to identify. Ultrasound examination is considered to have better accuracy than the Prader Orchidometer in estimating testicular size and evaluation of retrograde flow with Doppler mode [2].

The method most often used for the diagnosis of varicocele is an ultrasound examination of the scrotum because it is easy to use, and has high image resolution, can identify testicular vascular structure and flow, has high sensitivity and specificity. Usually, the examining physician will use ultrasound with a linear transducer>7.5 MHz with or without the Valsalva maneuver. One of the varicocele classifications commonly used in clinical practice is the Sarteschi classification as shown in: grade 1, defined as venous reflux that occurs during the valsalva maneuver and hypertrophy of the venous wall without stasis; grade 2, defined as supratesticular reflux during the valsalva maneuver with venous stasis without varicosities; grade 3, defined as, peritesticular reflux during the valsalva maneuver with early-stage cremasteric varicose veins; grade 4, defined as spontaneous basal reflux that increases with the valsalva maneuver accompanied by testicular hypotrophy and varicosities of the pampiniform plexus; and grade 5, defined as spontaneous basal reflux that does not increase with the valsalva maneuver accompanied by testicular hypotrophy and varicosities of the pampiniform plexus [2].

Varicocele is a form of varicose veins due to abnormal widening of the internal spermatic vein and the pampiniform venous plexus between the spermatic cord. Several pathophysiologies of varicoceles are associated with increased testicular temperature, increased venous pressure, oxidative stress, tissue hypoxia, and androgenic hormone imbalance. Varicocele is the most common cause of infertility in men, which contributes to 40% of cases with primary infertility and 80% with secondary infertility. The prevalence of varicocele cases in adolescents in a large population study shows a wide variation between 5-30%. Varicoceles were found in 25% of men with abnormal sperm analysis parameters and 12% with normal sperm analysis parameters [3].

The examination of varicocele in adolescence aims to prevent infertility in adulthood, but currently, no treatment and method can be used to predict it in the future. Physical examination is still the diagnostic standard and the presence of an ultrasound device provides greater convenience and accuracy when compared to an orchidometer [4]. Reduction of the size and volume of the testes on the same side (ipsilateral) and opposite (contralateral) to the position of the varicocele is usually detected by scrotal ultrasonography. In addition, with this tool, the dilation of the internal spermatic vein of more than 2-3 mm is usually used to establish the diagnosis of varicocele. Ultrasound with Doppler mode can also show venous return so that several important parameters can be measured such as venous reflux grade, peak retrograde flow, and hemodynamic pattern. However, assessment with scrotal ultrasound requires special skills and processing time which is highly dependent on operator experience [5].

Varicocele is one of the most common types of abnormalities that are often found in cases of infertility or subfertility in adult men. The pathophysiology of varicocele is mostly related to the condition of transient scrotal hyperthermia, the role of pro-inflammatory factors, and differences in protein expression in spermatozoa cells. In the structural abnormality of varicocele, heat stress occurs which affects the process of spermatogenesis, where the accumulation of reactive oxygen species will induce apoptosis or sperm cell death. The apoptotic process is mediated by decreased heat-shock protein (HSP), higher polymorphism of the glutathione S transferase gene and nitric oxide synthase gene, increased B-cell lymphoma associated X (BAX) expression and decreased expression of B-cell lymphoma protein 2 (BCl2) as a cell cycle guard. The process of stasis of blood flow in the varicocele veins will cause leukocyte cells to be trapped and activated by releasing reactive oxygen species (ROS) and hypoxia of testicular tissue occurs. This provides a profile of low-quality semen with abnormalities in sperm morphology, decreased sperm cell motility, reduced sperm cell count, and increased DNA abnormalities in sperm cells [6].

Another study mentions the role of the expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR1, VEGFR2, and VEGFR3) in varicocele cases in causing infertility in adult men. It is well known that VEGF is an important factor in the process of blood vessel formation (angiogenesis) where the overexpression of the VEGF/VEGFR axis in varicocele cases is significantly different from its expression in normal veins. It is thought to play a role in maintaining the survival of vascular endothelial cells in varicocele. In addition, VEGF also affects the decrease in the BAX/BCl2 ratio which is quite instrumental in reducing the process of spermatogenesis through the induction of the apoptotic pathway or sperm cell death [7]. In the case of varicocele, it is often found that the results of sperm analysis in the form of asthenozoospermia are associated with decreased sperm motility. One of the attempts to determine the etiology of these abnormalities is through metabolomics studies, which are defined as quantitative descriptions of all endogenous molecular metabolites between cells and fluids. Usually, these metabolomics studies are used for profiling metabolite products associated with a physiological or pathological condition. The study used the gas chromatography-mass spectrometry (GC-MS) method for the patient's sperm samples. Of the 33 types of metabolites that were identified in asthenozoospermia cases, 27 types of metabolites were decreased and 6 other types of metabolites were increased. In addition, in another similar study in the case of oligozoospermia, several metabolomic compounds were found with decreased levels, including aspartate, choline, fructose, and Myo-inositol. It can be used as a useful biomarker [8].

The development of electronic nose (e-nose) technology provides an opportunity to detect disease characteristics of volatile organic compounds (VOCs) produced by biological materials. Currently, metabolomics studies are widely used as a biomarker of male fertility, where all the changes that occur are associated with oxidative stress due to the formation of excessive ROS [9]. One of the applications of electronic nose technology is in the non-invasive detection of digestive tract diseases from the VOC headspace which produces a specific aroma for certain gastrointestinal diseases, where different metabolomic contents can be used as parameters to identify and distinguish between these diseases. These VOC gases come from a mixture of metabolites of normal flora bacteria and pathogenic bacteria, thus it is easier to take VOC samples from exhaled breath air samples with certain techniques [10].

Several things need to be considered when performing metabolomic profiling on semen samples, including the period of sexual abstinence before sampling. One study showed that short abstinence periods (2 days) were associated with lower secretion of metabolites by accessory glands and epididymis compared with longer abstinence periods (>2 days). In addition, it is known that the number of pyruvate and taurine metabolites per sperm count is associated with sperm motility [11]. In a study that identified differentially expressed proteins (DEPs) associated with infertility in unilateral varicocele cases compared with fertile donors, the results showed that downregulation of Kinesin family member 5B (KIF5B) and upregulation of Annexin A2 (ANXA2) have potential as potential biomarkers in cases of unilateral varicocele infertility. It is known that unilateral varicocele cases are the most common type of clinical case and this study used proteomic analysis to obtain these results [12].

However, there are not many studies that examine the potential of electronic nose technology to diagnose infertility, such as varicocele. This is related to the intrinsic factors of the sample and the appropriate sampling method for the electronic nose system. Another similar study was a study examining the metabolomic profile of urine samples in 64 prostate cancer patients and 51 benign prostatic hyperplasias (BPH) patients using 1H-nuclear magnetic resonance (1H-NMR). The comparative analysis used to differentiate the urine metabolomic profile was a multivariate statistical test. In the case of prostate cancer, the metabolomic characteristics that increased in concentration were branched-chain amino acids (BCAAs), glutamate, and pseudouridine. The metabolomic profiles whose concentrations decreased were glycine, dimethylglycine, fumarate, and 4-imidazole-acetate [13]. One of the ideas used in this study is to utilize metabolomic gases that form a pattern recognition of volatile organic compounds (VOCs) produced from the smell of sperm fluid. The supervised learning approach is used because the determination of varicocele disease requires data labeling from andrology specialists. In addition, because it aims to provide screening data that has high accuracy and sensitivity values as well as good detection speed, it is necessary to extract features from the data produced by gas sensors and a feature extraction process that is suitable for classification [14].

2. METHOD

This study was conducted by taking semen samples from patients with suspected infertility. The sample was obtained when the patient underwent a sperm analysis examination, which was then confirmed whether he had abnormalities in the form of varicocele or not through a physical examination and testicular ultrasound. We conducted this research from October to November 2021 in two hospitals with andrology clinics, namely Sadewa Hospital and Dr. Sardjito Hospital at Yogyakarta, Indonesia, and obtained 20 samples of semen. The semen sample obtained was then examined using an electronic nose, to be analyzed and classified using an artificial intelligence algorithm. As shown in Figure 1, we used a sperm sample of 0.3 ml in a 5 ml vac container tube, then using a 10 ml syringe connected to a plastic tube, the gas in the vac container tube is aspirated through a three-way stop cock and transferred to a 100 cc beaker tube with a modified lid with two holes. Then the electronic nose device is connected using a plastic hose, and data is collected for 60 seconds using data logger software. As for the data analysis and classification process, we use software with the Python programming language. The data processing using GeNose (electronic nose) is carried out in the physics and instrumentation laboratory, Universitas Gadjah Mada.

In this study, we use several types of values for feature extraction which are then followed by a comparison of supervised classification algorithms. We divided the study subjects into three groups, namely patients with non-varicocele, patients with unilateral varicocele, and patients with bilateral varicocele. We use five kinds of feature extraction values at 10th to 60th seconds data namely: gradient, max, min, mean, median, standard deviation, trapz and variance. Data preprocessing with minmax scaler on the dataset for data normalization. The stratified 3 fold cross-validation method is used during the machine learning process with the distribution of 30% testing dataset and 70% training dataset. The machine learning algorithms used are random forest (RF), k-nearest neighbors (kNN), support vector classification (C=1, gamma='scale', kernel= 'rbf'), and linear discriminant analysis (LDA). For data analysis, we use Python-based software.

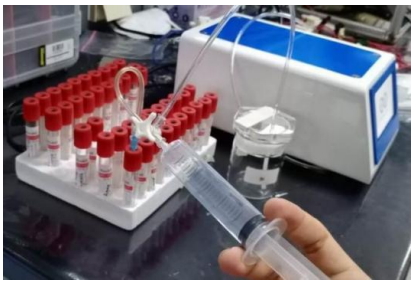

Figure 1. Method of examining seminal fluid samples using an electronic nose, beaker tube with a modified lid, the vac container tube, and modified syringe

3. RESULTS AND DISCUSSION

This research is a preliminary study to classify infertility with varicocele and non-varicocele using electronic nose technology. The characteristics of the research subjects in Table 1 show that the average age of the subjects of the preliminary study was 33.2 years with the largest group in the productive age range between 30-35 years. The length of storage of samples in the laboratory refrigerator is also quite varied with an average of 9.15 days, this is because it takes time to collect sufficient samples according to the schedule for examining the results of sperm analysis of patients in the fertility clinic laboratory. The abstinence period of the study subjects was also in accordance with the protocol for examining sperm analysis between 2-7 days with an average abstinence period of 4 days. The total volume of sperm fluid produced by the research subjects was quite varied with the average volume of semen being 3.72 cc and the largest group being 3-5 cc. Seminal fluid is a mixture of secretions from accessory glands and secretions from the testes, epididymis, and vas deferens. It is important to note in the analysis of the proteomic mixture of the seminal fluid is that the mixing process is not only influenced by the modulation of the expression of certain genes but is sometimes also influenced by the dilution process that occurs due to the relative contribution of other glands. In the case of varicocele experienced by adult men, proteomic studies are often associated with oxidative stress and inflammatory pathways. Moreover, it is related to the risk factors in heavy smokers which occur in the accumulation of toxins in the testes which of course changes the microenvironment in the process of spermatogenesis [15].

Table 1. Characteristics of varicocele and non-varicocele research subjects (20 subjects)

No	Parameters	Category	Amount	Average
1	Ages	< 30 years	6	33.2 years
		30 s.d 34 years	7	
		35 s.d 39 years	5	
		40 s.d 44 years	1	
		45 s.d 49 years	1	
2	Storage Time	< 3 days	6	9.15 days
		3 s.d 6 days	6	
		7 s.d 13 days	4	
		14 s.d 20 days	0	
		≥ 21 days	4	
3	Abstinence Period	< 4 days	9	4 days
		4 s.d 5 days	8	
		5 s.d 7 days	3	
4	Cement fluid volume	< 1 cc	0	3.72 cc
		1 s.d 2 cc	6	
		3 s.d 4 cc	11	
		5 s.d 6 cc	2	
		≥ 7 cc	1	
5	Categories of Varicocele	Non-Varicocele Clinical	7	Data labeling by an andrologist
		Unilateral varicocele	9	
		Bilateral Varicocele	4	

The data on the group of patients with clinical varicocele in Table 2 about the results of the ultrasound examination of the scrotum in this study indicate that more cases are unilateral (one-sided) varicocele cases compared to bilateral (two-sided) varicocele cases with some most of the cases were grade III to grade V. The descriptive data of the average testicular volume on the results of the scrotal ultrasound

examination showed testicular hypovolume in cases of varicocele. The information above may be related to a decrease in the amount of sperm production in varicocele patients. This is in line with previous studies that compared the total motile sperm count (TMSC) values obtained from patients with clinical varicocele and subclinical varicocele before and after varicocelectomy, where the difference was quite significant. It is known that the TMSC value is formed through three parameters, namely the volume of semen, sperm concentration, and sperm motility. These problems can be related to the occurrence of testicular hypovolume and the inflammatory process in varicocele cases [15].

Table 2. Results of scrotal ultrasound examination in varicocele patients (13 subjects)

No	Parameter	Category	Amount	Average testicular volume (cc)	
				Right	Left
1	Unilateral varicocele	Grade III	5	17.75	17.24
		Grade IV	1	17.58	13.47
		Grade V	3	18.94	15.23
2	Bilateral Varicocele	Grade III bilateral	1	32.08	29.29
		Mix Grade III dan Grade V	2	13.95	11.28
		Grade V bilateral	1	15.27	12.32

Most of the protein in the seminal fluid of sperm is obtained from the epididymis and prostasomes. The difference in expression of these exosomes greatly affects the case of azoospermia, asthenozoospermia, oligozoospermia, teratozoospermia, and other pathological conditions that affect male infertility. There are several candidate biomarkers of seminal fluid that have the potential to detect infertility in men with varicocele. Biomarkers whose expression is increased in varicoceles: Aldose reductase (ALDR), Cysteine-rich secretory protein-3 (CRISP3), Insulin-like growth factor binding protein-3 (IGFBP-3), Insulin-like growth factor binding protein-7 (IGFBP-7), Peroxiredoxin-1 (PRDX- 1), Peroxiredoxin-2 (PRDX-2), Polymeric immunoglobulin receptor (PIGR), and serine/threonine-protein kinase (SMG1). Biomarkers with decreased expression in varicoceles: Alpha-1-acid-glycoprotein 1 (ORM-1), Alpha-1-acid-glycoprotein 2 (ORM-2), Alpha-1-antitrypsin (SERPINA-1), Apolipoprotein D (APOD), Deoxyribonuclease-1 (DNAse -1), Glutathione hydrolase 1 proenzyme (GGT1), Prostatic specific acid phosphatase (ACPP), Semenogelin-1 (SEMG-1), and Semenogelin-2 (SEMG-2). Biomarkers whose expression is lost in varicoceles: Cysteine-rich secretory protein-1 (CRISP1), Thus proteomics in the seminal fluid can be used as a potential factor for diagnosis in a variety of different infertility cases [16].

The feature extraction of the mean, median, max, min, std, trapz and var values can be done using the dimensional reduction method using principal component analysis (PCA) with a total value of explained variance above 85%, as shown in Figure 2. In this classification study, we get a fairly good accuracy value for several algorithms (RF, kNN, support vector machine (SVM), latent dirichlet allocation (LDA)), both on training accuracy and on testing accuracy. The best results in this study showed an accuracy value of 0.83, precision 0.58 and recall 0.67 for several types of algorithms, as shown in Figure 3. These results are in line with a study examining metabolomic biomarkers of human semen for infertility cases. Another study showed that in infertile testes with varicocele there are several types of metabolomics whose levels are decreased or lower when compared to normal testes, including total choline (tCho), tCr, Myo-inositol (mI), Glx, and total lipids and macromolecules resonating. at 0.9 ppm (TLM09), 1.3 ppm (TLM13) and 2.0 ppm (TLM20) [17]. In addition, the development of electronic nose technology as an early detection tool for varicocele cases will greatly assist the prognosis of male fertility. The results of the varicocele screening will help analyze the need for varicoceleectomy in the future. In several studies, the varicocelectomy has a significant effect on the success of the Assisted Reproductive Technology method [18]–[20].

This study has several weaknesses, including in this study it does not make hormonal examinations as research parameters. In one study, it was stated that there was a significant difference in FSH levels between cases of varicocele with grade I-II and cases of varicocele with grade III-IV. FSH levels are elevated in cases of high-grade (III-IV) varicoceles [21]. In addition, this study did not consider elevated leukocyte levels in several subpopulations of patients with varicocele, which in one study showed higher leukocyte concentrations in varicocele patients [22]. It is known that a high leukocyte value can be caused by an obstructive inflammatory process in the epididymal tract and can also be caused by an infectious process in the genital tract. Thus it needs to be controlled further so as not to affect the gas metabolomic study of semen [23], [24]. Another thing that needs to be considered is the storage of semen, where previous studies comparing slow freezing and ultra-rapid freezing cryopreservation methods greatly affected the quality of stored sperm. In this study, sperm fluid was stored in a refrigerator and stored for a long time before being processed [25].

Figure 2. The visualization of 3D PCA (mean, median, max, min, std, trapz, var and grad) features extraction on varicocele dataset with a total value of explained variance above 85%

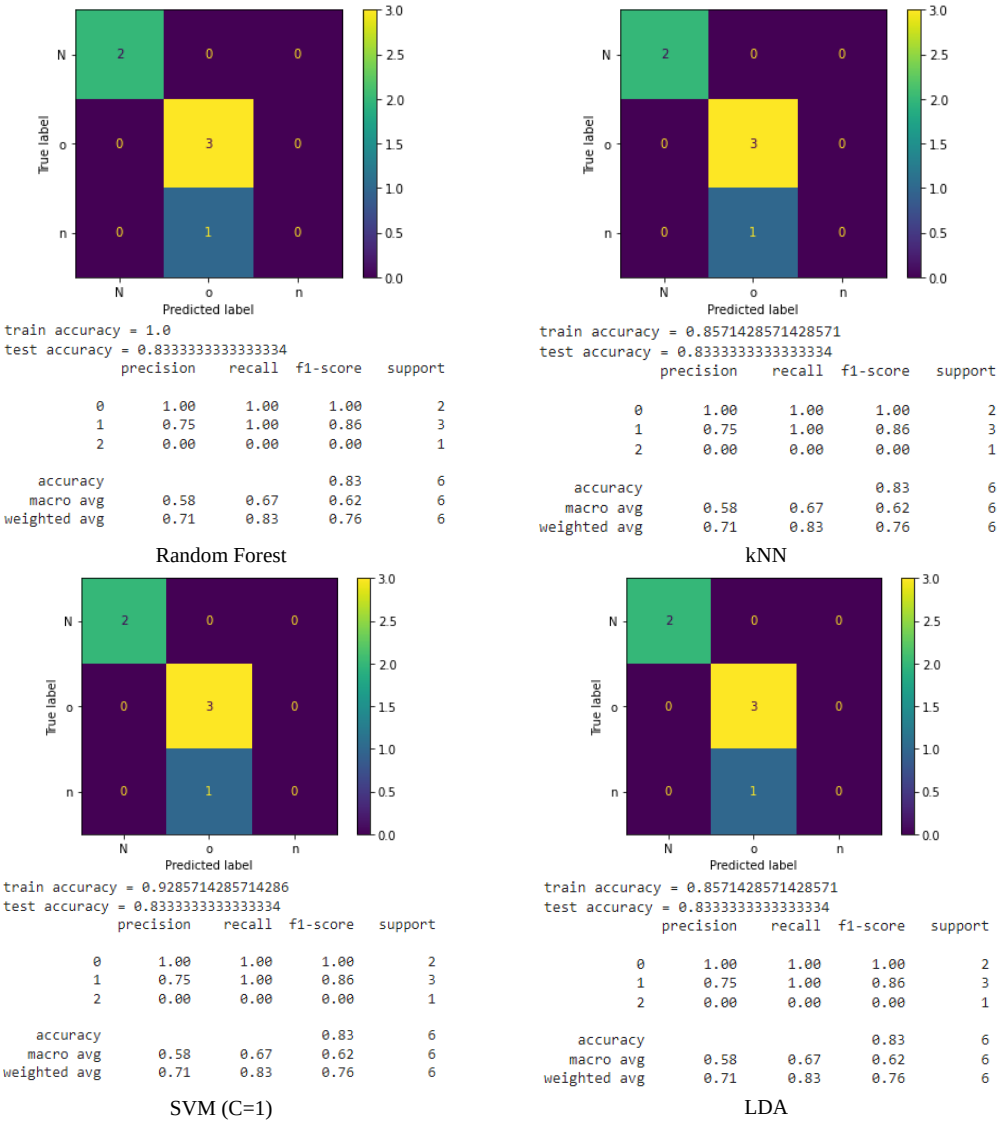

Figure 3. Comparison of the confusion matrix in the varicocele classification dataset using several algorithm (RF, kNN, SVM and LDA)

4. CONCLUSION

This initial study provides an overview of the potential of e-nose technology to be a diagnostic tool in cases of infertility due to varicocele. More sample subjects and experimental methods are still needed to get the best accuracy. For further research, deep learning method can be developed from the sensor pattern results in the data logger, thus the feature extraction process can be more optimal. The storage process, storage time, and bacterial contamination in semen samples also greatly affect the results of the analysis using an electronic nose.

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