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Research

Relationships between Smoking, Age, Body Mass Index, and Abstinence with DNA Fragmentation Index in Male Infertile Patients

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ABSTRACT

Background: Infertility increases annually, by 0.370% in women and 0.291% in men. One of the causes of male infertility is idiopathic in the form of cigarettes, alcohol, drugs, obesity, psychological stress, old age, dietary factors, and exposure to toxins in the environment or work. These factors can cause oxidative stress that can lead to sperm DNA damage or sperm DNA fragmentation as measured through the DNA Fragmentation Index (DFI). So this study to analyzed about the relationship between smoking, age, Body Mass Index, and abstinence period with DNA Fragmentation Index in infertile patient.
Subjects and Method: This type of research is observational analytics with a cross-sectional approach. The study was conducted at the Sekar Clinic of Dr. Moewardi Hospital Surakarta. A total of 35 subjects of male infertility patients performed DNA examinations of sperm fragmentation. Independent variables: smoking, age, body mass index (BMI), and abstinence. Dependent variable: DFI. Data is collected using medical records and analyzed using SPSS applications through univariate, bivariate, and multivariate analysis (logistic regression).
Results: Smoking (OR= 0.68; 95% CI= 0.52 to 2.23; p= 0.636), age (OR= 17.33; 95% CI=31.06 to 35.05; p= 0.003), BMI (OR= 1.47; 95% CI= 24.20 to 26.52; p = 0.58), abstinence (OR= 5.67, 95% CI =3.76 to 4.69; p=0.02). Results of multivariate analysis with logistic regression at age (OR= 13.62; 95% CI =1.42 to 130.60; p=0.024), and abstinence (OR= 3.94; 95% CI= 0.84 to 21.58; p= 0.114).
Conclusion: There is a relationship between age and abstinence to DFI. There is no relationship between smoking and BMI against DFI.

Keywords: smoking, age, body mass index, abstinence, DNA fragmentation index

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BACKGROUND

Infertility is a life crisis that affects couples from all over the world. Infertility has a negative impact on terms of psychosocial a person. Risk of depression, anxiety, and high distress in infertile patients (Rooney and Domar, 2018). Malefactors affect 50% of infertile couples worldwide (Khatun et al., 2018). About 30-50% of cases of male infertility are idiopathic, with no apparent cause or contribution to female infertility (Zeqiraj et al., 2018). Idiopathic factors such as

cigarettes, alcohol, drugs, obesity, psychological stress, old age, dietary factors, and exposure to toxins in the environment or work (Agarwal et al., 2021). These factors can cause oxidative stress that can damage sperm DNA (Barati et al., 2020). Sperm DNA fragmentation (SDF) can cause infertility, recurrent miscarriage, and birth defects (Bisht et al., 2017).

Smoking is a risk factor that has the potential to cause infertility. There are more than 7000 harmful chemicals in cigarette smoke (Durairajanayagam, 2018). The chemical in cigarette smoke is a Reactive Oxygen Species (ROS) that can reduce antioxidants. The buildup of ROS will cause oxidetive stress that will damage sperm function and ultimately interfere with male fertility (Harlev et al., 2015). Smoking is negatively associated with sperm count, motility, and morphology, and sperm DNA. Sperm DNA quality is worse in smokers than nonsmokers (Aboulmaouahib et al., 2018; Cui et al., 2016).

Body Mass Index (BMI) is a simple indicator used to measure the ratio of weight (kilograms) and height (meters) squared. Individuals with a higher BMI are at risk of obesity. Obesity signals poor lifestyle and food habits (Al Omrani et al., 2018). Obesity negatively impacts male fertility, including semen analysis parameters, sperm DNA integrity, and success in achieving pregnancy. In people with obesity, there are hormonal disorders, increased inflammatory mediators, increased testicular temperature, and the formation of ROS (Kahn and Brannigan, 2017).

Age is a contributing factor to irreversible infertility. But precisely according to the National Vital Statistics in America, every year there is a 40% increase in the age of father and mother to babies born due to delaying pregnancy. Some studies have mentioned Advance Paternal Age (APA) which is over the age of 40 years more negatively impacts sperm quality in the form of volume, motility, morphology, and increasing SDF (Brandt et al., 2019).

The world health organization (WHO) recommendation of the recommended abstinence period is 2-7 days. The American Society for Reproductive Medicine recommends abstinence in just 2-5 days. According to the American Urological Association, the Abstinence Period is only 2-3 days for infertile male evaluation. A prolonged abstinence period may increase sperm volume and number but sperm morphology motility decreases (Hanson et al., 2018). Shortening abstinence can significantly decrease DNA fragmentation (Kabukçu et al., 2021).

Based on WHO recommendations, sperm analysis remains a routine diagnostic test for male infertility (Cho and Agarwal, 2018). However, sperm analysis examination only assesses physical characteristics (e.g., color, volume, pH, smell, viscosity, and thawing time), sperm concentration, motility, development, viability, morphology, and leukocytes. Additional tests are needed to evaluate a man's fertility potential (Softness et al., 2020). One solution can be done by doing a sperm DNA examination. SDF examination can measure the quality of sperm as carriers of DNA genes, making it more significant than previous sperm analysis parameters (Kim, 2018). SDF damage adversely affects fertilization and embryo development. Infertile men have greater sperm DNA damage than fertile men. Embryos derived from SDF may increase the risk of genetic diseases in offspring (Agarwal et al., 2019). So that SDF examination is needed to find out whether smoking factors, age, BMI, and abstinence period cause sperm damage presented through the DNA Fragmentation Index (DFI). This aim of the study to analyzed about the relationship between smoking, age, Body Mass Index,

and abstinence period with DNA Fragmentation Index in infertile patients.

SUBJECTS AND METHOD

1. Study Design

Analytical observational studies with a cross-sectional approach. The study was conducted from September to November 2021 the Sekar Clinic of Dr. Moewardi Hospital.

2. Population and Sample

The study subjects were male infertility patients the Sekar Clinic of Dr. Moewardi Hospital who had conducted SDF examinations.

3. Study Variables

The free variables of the study were smoking, age, BMI, and abstinence. The bound variable of the study is DFI.

4. Operational Definition of Variables

Smoking, defined as the behavior of inhaling tobacco smoke burned through cigarettes, pipes, or cigars. Category scale, for data analysis, changed dichotomy 0= Smoking, 1= No smoking.

Age is the length of a person's life from birth to a certain moment (SDF examination). Category scale, for data analysis, changed dichotomy 0= >35 years, 1= ≤35 years.

Body Mass Index is a standard anthropometric calculation by comparing weight and height (Nuttall, 2015). Category scale, for data analysis, changed dichotomy 0= ≥25 kg/m^2 (Not normal), 1= <24.9 kg/m^2 (Normal).

Abstinence, the period when men do not secrete sperm fluid either in the form of sexual activity, masturbation, or wet dreams for a minimum of two days maximum of seven days (Hanson et al., 2018). Category scale, for data analysis, changed dichotomy 0=5, 6, 7 days, 1= 2, 3, 4 days.

DNA Fragmentation Index is the result of DNA Fragmentation Sperm examination obtained by comparing the total spermatozoa DNA damaged with the total spermatozoa observed, results in percentage form (Lestari and Sari, 2015). Category scale, for data analysis is changed to dichotomy 0= >26.1% (abnosrmal DFI), 1= ≤26.1% (normal DFI).

5. Study Instruments

The instrument used is secondary data of medical records of patients undergoing SDF examination and sperm analysis at the Fertility Clinic Sekar Dr. Moewardi Hospital Surakarta.

6. Data analysis

Univariate, bivariate and multivariate analysis using logistic regression analysis Data processing used IBM SPSS 26 applications.

7. Research Ethics

Ethical research includes informed consent, anonymity, and ethical clearance. Ethical clearance was approved by the Research Ethics Committee at Dr. Moewardi Hospital, Surakarta, Indonesia, No. 932/IX/HREC/-2021, on 06 October 2021.

RESULTS

1. Sample Characteristics

Obtained research subjects as many as 35 people, with the characteristics of the research subject as listed in Table 1. Most subjects did not smoke as many as 26 subjects with a percentage of 74.30%. Most of them are >35 years old with 22 subjects with a percentage of 62.90%. Abnormal BMI has obtained as many as 18 subjects with a percentage of 51.40%. Most of the abstinence period of the subject is 2,3,4 days as many as 23 subjects with a percentage of 65.70%. Abnormal DFI has obtained as many as 18 subjects with a percentage of 51.40%.

Table 1. Characteristics of The Research Subject

Characteristic	n	%
Smoking		
Smoking	9	25.70
No Smoking	26	74.30
Age (years)		
>35	22	62.90
≤35	13	37.10
BMI (kg/m²)		
No normal	18	51.40
Normal	17	48.60
Abstinence (days)		
5,6,7	12	34.30
2,3,4	23	65.70
DFI		
No normal	18	51.40
Normal	17	48.60

2. Bivariate Analysis

Table 2. Relationship between Smoking, Age, BMI, and Abstinence with DNA Fragmentation Index

Variable	DFI				Total		OR	95% CI		p
	Normal		No Normal					Lower limit	Upper limit	
	n	%	n	%	n	%				
Smoking										
Smoking	6	28.60	3	24.40	9	25.70	0.68	0.52	2.23	0.636
No Smoking	15	71.40	11	78.60	26	74.30				
Age (years)										
> 35	9	42.90	13	92.90	22	62.90	17.33	31.06	35.05	0.003
≤ 35	12	57.10	1	7.10	13	37.10				
BMI (kg/m²)										
No Normal	10	47.60	8	57.10	18	51.40	1.47	24.20	26.52	0.581
Normal	11	52.40	6	42.90	17	48.60				
Abstinence (days)										
5,6,7	4	19.00	8	57.10	12	51,40	5.67	3.76	4.69	0.024
2,3,4	17	81.00	6	42.90	23	65.70				

Based on table 2, it can be seen that there is no relationship between smoking and DFI, p= 0.636 and OR= 0.68. Men who smoked had an abnormal likelihood of DFI by 0.68 times that of men who did not smoke. There is a relationship between age and DFI, p= 0.030 and OR= 17.33. Age >35 years have an abnormal possibility of DFI of 17.33 times compared to the age of ≤35 years. There is no relationship between BMI and DFI, p= 0.581 and OR= 1.47. Men with abnormal BMI had an abnormal chance of DFI of 1.47 times compared to men with normal BMI. There is a relationship between abstinence and DFI, p= 0.020 and OR= 5.67. Men with an abstinence period of 5,6,7 days had an abnormal chance of

DFI of 5.67 times compared to men with abstinence at 2,3,4 days.

Table 3. Results of Logistic Regression Analysis on the Relationship of Age and Abstinence With DFI

Variable	SE	OR	95% CI		p
			Lower	Upper	
Age (>35 years)	1.153	13.62	1.42	130.60	0.024
Abstinence (5,6,7 days)	0.868	3.94	0.84	21.58	0.114
n= 35					
Log likehood = 34.198					
p < 0.001					
R^2 = 41.7%					

Based on table 3 of logistic regression analysis on the relationship of age and abstinence with DFI, the most influential is age (OR= 13.62; 95% CI= 1.42 to 130.60; p= 0.024). That is, the age of >35 years is likely to have an effect of 13,621 times increase abnormal DFI compared to the age of ≤35 years. Abstinence does not have a significant effect on DFI because it has a value (OR= 3.94; 95% CI= 0.84 to 21.58; p= 0.114). That is, although the effect of abstinence on DFI is meaningless, the longer abstinence period has a possibility of 3.94 times increasing abnormal DFI compared to shorter abstinence periods. Negelkerke R Square of 0.417 which means the influence of both free variables (age and abstinence) is jointly related to sperm DFI by 41.70% the remaining 58.30% is influenced by other variables not observed in this study.

DISCUSSION

1. Smoking relationship with DFI

Statistically, there was no significant association between smoking and DFI. This study does not support the research conducted by Durairajanayagam, (2018) mentions that the chemicals in cigarette smoke are ROS that can reduce antioxidants. The buildup of ROS will cause oxidative stress that will damage sperm function and ultimately interfere with male fertility (Durairajanayagam, 2018). Other studies have also mentioned that nicotine contained in cigarettes can cause sperm cell death and sperm DNA damage due to the formation of excess ROS. Excess ROS will inhibit antioxidant molecules that function in repairing sperm DNA damage.

The accumulation of nicotine in the blood can interfere with the regulation of the pituitary-gonadal axis hypothalamus by increasing apoptosis during spermatogenesis, thereby causing more damage to sperm DNA that is more oxidative (Anifandis et al., 2014). Research conducted by Aboulmaouahib et al., (2018); Cui et al. (2016) Smoking has been negatively associated with sperm count, motility, and morphology, and sperm DNA. Sperm DNA quality is worse in smokers than nonsmokers.

2. Relationship of age with DFI

The results of the study statistically showed a significant association between age and DFI. Age >35 years may have an effect of 17.333 times increase abnormal DFI compared to age ≤35 years. Elderly men's sperm have greater DNA fragmentation than younger men's sperm (Sigman, 2020). Aging is associated with reduced expression of nucleus 8-oxoguanine DNA glycosylase OGG1 which has an impact on increasing OGG1 expression in mitochondrial DNA causing lesions on sperm DNA (Bisht et al., 2017).

Brandt et al., (2019) In their research

mentioned that at the age of over 40 years there is a decrease in the quality of gametes and the quality of semen triggered by the accumulation of mutations in the nucleus and mitochondria of sperm. Accumulation of mutations in sperm DNA results in increased cell division reduced DNA replication, inefficient DNA repair, and accumulation of mutagens from external and internal sources, including from exposure to oxidetive stress (Agarwal et al., 2017).

As testicular function ages and the body's metabolism in cell repair deteriorates (Durairajanayagam, 2018). Morphological changes related to testicular age include decreased number of germ cells, Leydig and Sertoli cells, as well as structural changes, including narrowing of seminiferous tubule cells. In old age, there is a decrease in the capacity to repair cellular and tissue damage from exposure to toxins or diseases (Albani et al., 2019). Along the course of aging, the regulation of the hypothalamic-pituitary-gonad axis changes so that apoptosis or accumulation of ROS in male germ cells can occur. These events can cause oxidative stress and disruption to sperm DNA.

3. Relationship of BMI with DFI

Based on the results of the study statistically, there is no significant association between BMI and DFI. This is in line with the research (Bandel et al., 2015) that overweight has not been able to affect sperm DNA damage in the form of sperm DNA fragmentation. But if obesity greatly affects sperm DNA damage.

According to (Kahn and Brannigan, 2017), Obesity negatively impacts male fertility, including semen analysis parameters, sperm DNA integrity, and success in achieving pregnancy. In people with obesity, there are hormonal disorders, an increase in inflammatory mediators, an increase in testicular temperature, and the formation of ROS. This occurs because excess adipose tissue causes the conversion of testosterone to estrogen, consequently inhibiting the hypothalamic-pituitary-gonad axis, which has a negative effect on spermatogenesis. Excessive oxidative stress and pituitary-hypothalamic dysregulation occur due to increased conversion of androgens to estrogen, thereby increasing SDF (Le et al. 2020).

The impact of obesity is very bad on male fertility because it causes an increase in several factors such as SDF, scrotal hyperthermia, erectile dysfunction, germ cell apoptosis, changes in deteriorating sperm analysis parameters, which eventually lead to male infertility (Leisegang et al., 2021). Obesity is also considered a pro-inflammatory state that causes an increase in systemic inflammation. Intake of foods containing saturated fatty acids can increase the formation of ROS and disrupt the epigenetic status of sperm (McPherson and Lane, 2015).

4. Abstinence relationship with DFI

The results of the study statistically showed a significant association between abstinence and DFI. Longer abstinence periods are 3.94 times more likely to increase abnormal DFI than shorter abstinence periods. The short abstinence period is beneficial because the DNA integrity of sperm will be at optimal levels. The antioxidant reserves in sperm plasma are at their maximum level, and sperm analysis parameters are within normal limits (Agarwal et al., 2016; Pons et al., 2013). So some studies recommend the use of short abstinence periods because it can increase sperm quality rather than quantity (Dupesh et al., 2020).

There are some limitations on this study such as cannot perform standard anthropometric measurements because of secondary data, use BMI not abdominal and hip circumference (more accurate in determining obesity), other factors that need to be investigated (varikokel, psycologist stress, food factors,toxin environment or occupa-

tion of infetil patient), and not perform hormonal tests such as FSH and testosterone on research subjects.

AUTHOR CONTRIBUTION

Atifa Nadira Ediningtyas, Abdurahman Laqif, Eriana Melinawati and Supriyadi Hari Respati together choose topics, explore, collect data and analyze data.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest in the process of this research.

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