The Relationship between Seminal Total Antioxidant Capacity and Idiopathic Repeated Pregnancy Loss

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Abstract

Background: Attempts to demonstrate a link between idiopathic recurrent pregnancy loss (RPL) and sperm quality have been contentious. Based on this debate, other factors such as the antioxidant capacity of seminal plasma and the amounts of free oxygen radicals known as seminal reactive oxygen spices (ROS) should be considered.

Objective: Evaluation of the role of seminal total antioxidant capacity (TAC), in idiopathic recurrent pregnancy loss.

Methods: One hundred men were enrolled. Wife of these men had undergone full gynecologic evaluation and basic investigations of infertility and recurrent pregnancy loss of them were normal. Cases were divided into two groups; group 1 "Recurrent pregnancy loss (RPL) group" composed of 50 male partners of couples who had previously experienced at least two clinical first trimesteric idiopathic miscarriages and group 2 "Control group"; composed of 50 fertile men, with at least one child without assisted reproduction treatments, normal karyotype, normal sperm parameters, and wives who had no history of miscarriage.

Results: Seminal total antioxidant capacity was significantly lower in RPL group; 1.25 ± 0.36 vs. 1.75 ± 0.64, $p<0.001$. Also, there was no significant correlation between seminal total antioxidant capacity and paternal age, BMI and seminal fluid examination. On the other hand, there was positive correlation between it and number of previous abortions. Regarding performance of seminal total antioxidant capacity to predict cases with RPL; statistical analysis of current results showed that AUC was 0.741 (95% confidence interval: 0.641-0.842). At a cut-off point > 1.46, the sensitivity was 70% and specificity was 64%.

Conclusion: Seminal total antioxidant capacity is a major contributor to idiopathic repeated pregnancy loss. At cutoff point > 1.46; it had sensitivity 70% and specificity 64% in prediction of cases of RPL.

Keywords: Seminal; Antioxidant Capacity; Repeated Pregnancy Loss

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INTRODUCTION

Based on the World Health Organization (WHO) guidelines, miscarriage has been defined as the loss of an embryo weighing 500g or less that occurs before 20 weeks of gestation and may vary with age and parity of the mother. Repeated pregnancy loss (RPL) is the loss of two or more consecutive clinical pregnancies in the first trimester of gestation. One percent of couples experience recurrent pregnancy miscarriage [1].

The main known etiological causes for RPL include uterine anatomical anomalies, genetic factors or chromosomal abnormalities, and infectious, immunological and endocrine disorders. Conversely, 40%−50% of RPL cases have no identified cause and are therefore classified as unexplained or idiopathic. Although pregnancy miscarriage is related to women, it is possible that the male partner has a role in these idiopathic cases. Since 50% of the embryonic chromosomes are paternal, and the male gamete contributes in placental and embryonic development so it is rational to analyze semen parameters to determine their role in idiopathic RPL [2].

It should be noted that routine semen analysis reveals only information about the concentration, motility and morphology of the sperm and does not provide information regarding sperm functional competence. Attempts to show a relationship between idiopathic RPL and sperm quality, evaluated through conventional internationally established guidelines for semen analyses, have been controversial. Based on this controversy, it is important to take into account other parameters, such as the antioxidant capacity of seminal plasma and the levels of free oxygen radicals known as seminal reactive oxygen spices (ROS) [3].

It is known that human spermatozoa generate reactive oxygen species ROS in physiologic amounts, which play a role in sperm functions during sperm capacitation, acrosome reaction, and oocyte fusion. However, uncontrolled and excessive production of ROS, when it overwhelms the antioxidant defenses in semen, results in seminal oxidative stress and sperm damage. Recently, a substantial body of growing evidence suggests that such seminal oxidative stress is involved in many cases of idiopathic RPL [4].

As it has been demonstrated that reactive oxygen species (ROS) can be detected and measured in human spermatozoa. Superoxide and hydrogen peroxide (H2O2) are the common forms of reactive oxygen species (ROS). They can interact with nearby molecules and thus play a key role in inducing sperm damage and even further damage of early gestation that may be involved in causing multiple or idiopathic loss of gestation [5].

The reduced ability of seminal reductive enzymes or reduced its amounts of antioxidants is called recently as Total antioxidant capacity (TAC) which is considered also as physiologic function of seminal plasma that maintains the balance between oxidation and reduction processes during sperm capacitation, acrosome reaction, and oocyte fusion. It has been demonstrated that the TAC can be useful as predictor for genetic alteration and sperm quality. In this study, relationship between amount of free radicals, and total antioxidant capacity (TAC) in semen have been considered as a risk factor for spontaneous miscarriage hypothesis [6].

METHODS

One hundred men were enrolled.

Study type

It was a case control study.

Study place

The study was conducted at Shebin El Kom infertility clinic (Benha University Hospital).

Study period

The study was conducted from February 2020 until February 2021.

Inclusion criteria

Couples had a history of at least two idiopathic first trimesteric miscarriages. Wife of these men had undergone full gynecologic evaluation and basic investigations of infertility and recurrent pregnancy loss and all of them were normal.

Exclusion criteria

Wives aged over 35 years, had gestational age more than 3 months or with known factors of RPL as sub mucous fibroid, congenital uterine malformation, sever intrauterine (IU) synechia, cervical incompetence, uncontrolled DM, hypothyroidism, antiphospholipid antibody syndrome, hydro-salpinx, PCO, endometriosis, hyper-prolactinemia or obesity. Also, husbands with a history of exposure to environmental or occupational toxicants as heavy metal or radiation exposure with proven toxicity on fertility or using medication with proven toxicity on fertility, infertility secondary to infections as orchitis due to mumps and sexually transmitted diseases, congenital defects as epididymis or vas deferens alterations and inguinal surgery.

Study procedures

This study was approved by the Committee for Clinical Research at Benha University Hospital and informed written consent was obtained from all participants. Additional clinical information were extracted from the records of each patient.

A complete medical history and clinical examination were performed for every patient. Their medical charts were reviewed for age, body mass index (BMI), primary or secondary infertility, and duration of infertility. A history of antioxidant and/or antibiotic prescription use was be also verified. Cases were divided into two groups:

- Group 1 "Recurrent pregnancy loss (RPL) group"; composed of 50 male partners of couples who had previously experienced at least two clinical first trimesteric idiopathic miscarriages.
- Group 2 "Control group"; composed of 50 fertile men, with at least one child without assisted reproduction treatments, normal karyotype, normal sperm parameters, and wives who had no history of miscarriage.

Semen Samples:

Semen samples were obtained by masturbation after 72 hours of sexual abstinence. After complete liquefaction of the sample, semen analysis was performed according to World Health Organization guidelines 2010.

Determination of total antioxidant capacity By TAC Kit

The determination of the antioxidative capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H2O2). The antioxidants in the sample eliminated a certain amount of the provided hydrogen peroxide. The residual H2O2 was determined colorimetrically by an enzymatic reaction which involved the conversion of 3, 5, dichloro –2– hydroxybenzensulphonate to a coloured product.

STATISTICAL ANALYSIS

All data were coded and analysed using the computer program SPSS (Statistical package for social science) version 23.0 to obtain descriptive data. Descriptive statistics were calculated.

RESULTS

There was statistical significant difference between RPL and control groups regarding age of wife and husband, while there was no significant difference between groups regarding duration of marriage (Table 1)

Table 1: Comparison between the studied groups regarding partners age and duration of marriage

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
Age of wife / years	**Mean ±SD**	32.4±4.3		28.2±2.5		t=5.9	<0.001*
	Range	26-41		23-34			
Age of husband /years	**Mean ±SD**	36.3±4.9		32.8±3.4		t=4.1	<0.001*
	Range	30-49		27-42			
Duration of marriage / years	**Mean ±SD**	7.7±2.7		7.4±3.3		t=0.56	0.57
	Range	4-13		2-15			

There was statistical significant difference between RPL and control groups regarding previous complete pregnancy and mode of delivery (Table 2)

Table 2: Previous complete pregnancy and mode of delivery in the studied groups

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
Previous complete pregnancy	0	24	48.0%	0	0.0%	X^2=65.4	<0.001*
	1	25	50.0%	11	22.0%		
	2	1	2.0%	14	28.0%		
	3	0	0.0%	18	36.0%		
	4	0	0.0%	3	6.0%		
	5	0	0.0%	4	8.0%		
Mode of delivery	C/S	26	100.0%	33	66.0%	X^2=11.3	<0.001*
	Vaginal	0	0.0%	17	34.0%		

There was statistical significant difference between RPL and control groups regarding previous abortion (Table 3).

Table 3: Previous abortion in the studied groups

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
Previous abortion	0	0	0.0%	50	100.0%	X^2=88.9	<0.001*
	1	0	0.0%	0	0.0%		
	2	7	14.0%	0	0.0%		
	3	23	46.0%	0	0.0%		
	4	19	38.0%	0	0.0%		
	5	1	2.0%	0	0.0%		

There was no significant difference between RPL and control groups regarding clinical assessment of females (Table 4).

Table 4: Clinical assessment of females in the studied groups

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
BMI	**Mean ±SD**	25±2.3		25.7±3.2		t=1.59	0.115
	Range	22-33.5		22.9-31.5			
Medical history	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
Gynecological history of pain , bleeding , discharge	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
Possible causes of RPL History	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
General examination	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
Local examination	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		

There was statistical significant difference between RPL and control groups regarding clinical assessment in males (Table 5).

Table 5: Clinical assessment of males in the studied groups

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
BMI	**Mean ±SD**	24.5±2.3		24.7±2.8		t=1.39	0.173
	Range	21-35		21.9-35			
Smoking	**Yes**	20	40.0%	19	38.0%	X^2=0.044	0.84
	No	30	60.0%	31	62.0%		
History genital surgery	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
Exposure to toxicants of proven effect on fertility	**Yes**	0	0.0%	0	0.0%	-	-
	No	50	100.0%	50	100.0%		
General examination	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		
Local examination	**Positive**	0	0.0%	0	0.0%	-	-
	Free	50	100.0%	50	100.0%		

The mean volume of semen of RPL group was 4 ml, the mean liquefaction time was 20 min., the mean total sperm count was 46, the mean progressive motility was 42.4%, the mean abnormal shapes was 81.6%, and the mean vitality was 55.98%. (Table 6).

Table 6: Seminal fluid analysis in RPL group

	RPL group				
	Mean	**±SD**	**Median**	**Min.**	**Max.**
Volume /ml	4	1	4	2	5
Liquefaction time/ min.	20	1	20	16	23
Total sperm count/ mil	466	15	44	23	90
sperm concentration/ mil	46	15	44	23	90
Progressive motility %	42.44	7.81	43.00	28.00	57.00
Abnormal shape %	81.60	11.37	86.50	55.00	97.00
Vitality %	55.98	10.59	56.00	39.00	78.00

There was statistical significant difference between RPL and control groups regarding seminal total antioxidant capacity, as it was significantly lower in RPL group (Table 7).

Table 7: Seminal total antioxidant capacity in the studied groups

		RPL group		Control group		Test	P value
		N=50	%	N=50	%		
Seminal total antioxidant capacity	**Mean ±SD**	1.25±0.36		1.75±0.64		t=4.8	<0.001*
	Median	1.66		1.2			
	Range	0.32-2.97		0.32-1.95			

there was no significant correlation between seminal total antioxidant capacity and (Age, BMI, seminal fluid examination) (Table 8).

Table 8: Correlation between seminal total antioxidant capacity and clinical data in RPL group

		Seminal total antioxidant capacity	
		r	**P value**
Age/ years		-0.021	0.837
BMI		0.024	0.811
Seminal fluid examination	**Volume /ml**	0.107	0.467
	Liquefaction time/ min	0.067	0.599
	Total sperm count/ mil	0.028	0.845
	Sperm concentration/ mil	0.028	0.845
	Progressive motility %	0.159	0.270
	Abnormal shape %	-0.035	0.808
	Vitality %	0.099	0.493

There was statistical significant difference in seminal total antioxidant capacity, as regards number of previous complete pregnancy (Table 9).

Table 9: Seminal total antioxidant capacity according to previous complete pregnancy

		Seminal total antioxidant capacity				Test	P value
		Mean	±SD	Min.	Max.		
Previous complete pregnancy	0	1.75	0.67	0.75	2.97	F=3.1	0.010*
	1	1.60	0.62	0.32	2.79		
	2	1.28	0.38	0.32	1.73		
	3	1.27	0.37	0.75	1.95		
	4	1.43	0.15	1.26	1.55		
	5	0.94	0.11	0.79	1.06		

There was statistical significant difference in seminal total antioxidant capacity, as regards number of previous abortion (Table 10).

Table 10: Seminal total antioxidant capacity according to previous abortion

		Seminal total antioxidant capacity				Test	P value
		Mean	±SD	Min.	Max.		
Previous abortion	0	1.25	0.36	0.32	1.95	F=7.98	<0.001*
	2	2.14	0.75	1.13	2.97		
	3	1.71	0.62	0.32	2.79		
	4	1.61	0.60	0.75	2.63		
	5	2.49	.	2.49	2.49		

ROC analysis was done to assess the performance of Seminal total antioxidant capacity to detect cases with RPL; AUC was 0.741 (95% confidence interval: 0.641-0.842), p<0.001. At a cutoff point > 1.46, the sensitivity was 70% and specificity was 64% (Table 11).

Table 11: Performance of Seminal total antioxidant capacity to predict cases with RPL

	AUC	95% CI		Cut-off value	Sensitivity	Specificity	P value
Seminal total antioxidant capacity	0.741	0.641	0.842	>1.46	70%	64%	<0.001*

DISCUSSION

Current study disagreed with Kamkar and his colleague who conducted a case-control study that comprised 42 couples who had experienced idiopathic RPL and 42 fertile men as the control group. They stated that sperm motility in the patients was significantly less than the control group (P=0.001). On the other hand, they agreed with us and stated that the sperm count and morphology was not significantly different between the two studied groups [1].

Fazeli and Salimi aimed to determine the correlation between total antioxidant capacity (TAC) and malondialdehyde (MDA) as markers of oxidative stress in relation to idiopathic male infertility and sperm parameters. This case control study was conducted using 35 men with idiopathic infertility and 34 men with proven fertility. Seminal plasma TAC and MDA were measured by ferric reducing ability of plasma (FRAP) and thiobarbituric acid (TBA) reaction methods, respectively. They corresponded with current study and stated that seminal TAC levels were signifi-

cantly lower and seminal MDA levels were significantly higher in men with idiopathic infertility than in fertile men (P<0.0001 and P=0.004, respectively) [7].

Vatannejad and his colleague conducted a study to evaluate reactive oxygen species (ROS), total antioxidant capacity (TAC) and ROS-TAC score as indicator for oxidative stress status as well as 8-hydrodeoxyguanosine (8-OHdG) levels as a marker for DNA damage in the seminal plasma of asthenozoospermia patients compared to normozoospermia samples, however our study assessed TAC in normozoospermia samples only. They disagreed with current study and stated that no significant difference was observed in TAC levels between the groups. ROS-TAC score in astheno¬zoospermic men was lower than normozoospermic men (P = .02) [8].

Current study agreed with Kamkar and his colleague who stated that the total antioxidant capacity was 2.69 ± 0.88 in the RPL group compared to 3.63 ± 1.31 nm in the controls, respectively [1].

Huang and his colleagues conducted a systematic review and meta-analysis of observational case-control studies to evaluate markers of oxidative stress in seminal plasma of patients with male infertility. They agreed with current study and stated that the concentrations of GSH (SMD = –1.68, p < 0.00001), vitamin C (SMD = –1.12, p < 0.00001), and vitamin E (SMD = –1.48, p = 0.003), as well as the activities of catalase (SMD = –1.91, p < 0.0001), glutathione peroxidase (SMD = –1.96, p = 0.0002) and glutathione-S-transferase (SMD = –1.62, p = 0.009) declined remarkably, resulting in decreased total antioxidant capacity (SMD = –1.77, p < 0.00001) [9].

Twenty infertile men (test group) attending various fertility centers were recruited in the study and 20 fertile male volunteers (control group) were also recruited as procedural controls based on the biophysical analysis of semen in a study conducted by Riaz and his colleagues. They were in line with current study and stated that TAS concentration was lower in the infertile group compared to the fertile group. The results were shown as mean ± SD (95% CI), 0.65 ± 0.29 (0.51-0.79) vs. 0.98 ± 0.34 (0.82-1.14) respectively [10].

In a cross sectional study conducted by Issa Layali and his colleagues, 59 semen samples were provided by fertile (n=12) individuals as control, infertile patients with normal viscosity (n=25) and infertile patients with hyper viscosity (n=22). Seminal plasma TAC was measured by ferric reducing of antioxidant power (FRAP). They agreed with us and stated that the mean of seminal plasma TAC value in seminal plasma of non-hyper viscosity patients (1710.31 ± 458.67 µmol/l) was significantly (p<0.01) higher than that of hyper viscosity group (1230.25 ± 352 µmol/l) [11].

Fazeli and Salimi disagreed with current study and stated that a positive correlation was shown between sperm motility, sperm morphology, and TAC levels in men with idiopathic infertility (P=0.002 and P=0.002, respectively). In addition, there was a correlation between sperm motility and TAC levels in fertile men (P=0.005). There was no correlation between sperm count and TAC levels in either men with idiopathic infertility or in fertile men [7].

Same to current study, seminal plasma from 279 infertile patients and 46 normal healthy men referred to a male infertility testing laboratory were tested to measure TAC by a colorimetric assay kit in a study conducted by Roychoudhury and his colleagues. Infertile patients showed significantly lower levels (mean ± SEM) of total antioxidants (micromolar Trolox equivalents) in their seminal plasma (1863.84 ± 27.16 µM) compared to those from fertile men (2013 ± 56.04 µM, P = 0.019). A preferred cutoff TAC value of 1947 µM could facilitate better diagnosis of oxidative stress (OS) in men with male factor infertility. At this threshold, the specificity of TAC assay was 63.0 % and the sensitivity 59.5 % with a positive predictive value of 90.7 % and a negative predictive value of 20.4 % 12.

Strengths

The strengths of current study were due to every effort was made to ascertain that all data were documented, and only complete information was included in data analysis. All clinical evaluations and assessment of study outcomes were done by the same team.

Limitations

The limitations of current study were due to COVID 19 pandemic and relatively small sample size regarding accuracy of study outcomes.

CONCLUSION

Seminal total antioxidant capacity (TAC) is a major contributor to idiopathic repeated pregnancy loss (RPL). Seminal total antioxidant capacity is significantly lower in RPL patients. There was positive correlation between TAC and number of previous abortions. TAC at cutoff point > 1.46 had sensitivity 70% and specificity 64% in prediction of cases of RPL.

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REFERENCES

1. Kamkar N, Ramezanali F, Sabbaghian M. The relationship between sperm DNA fragmentation, free radicals and antioxidant capacity with idiopathic repeated pregnancy loss. Reproductive Biology. 2018 .18(4):330-5.
2. Wu Z, You Z, Zhang C, Li Z, Su X, Zhang X, et al. Association between functional polymorphisms of Foxp3 gene and the occurrence of unexplained recurrent spontaneous abortion in a Chinese Han population. ClinDevImmunol 2016;89:6458-62
3. Amaral A, Castillo J, Estanyol JM, Ballescà JL, Ramalho-Santos J, Oliva R. Human sperm tail proteome suggests new endogenous metabolic pathways. Molecular & Cellular Proteomics. 2013; 12(2):330-42.
4. Jiang H, He RB, Wang CL, Zhu J. The relationship of sperm DNA fragmentation index with the outcomes of in-vitro fertilization-embryo transfer and intracytoplasmic sperm injection. Journal of Obstetrics and Gynecology. 2011; 31(7):636-9.
5. Darszon A, Nishigaki T, Beltran C, Treviño CL. Calcium channels in the development, maturation, and function of spermatozoa. Physiological reviews. 2011; 91(4):1305-55.
6. Agarwal A, Sharma RK, Sharma R, Assidi M, Abuzenadah AM, Alshahrani S, et al. Characterizing semen parameters and their association with reactive oxygen species in infertile men. ReprodBiolEndocrinol 2016, 12:33-37
7. Fazeli, F., &Salimi, S. Correlation of seminal plasma total antioxidant capacity and malondialdehyde levels with sperm parameters in men with idiopathic infertility.2016,4:29736
8. Vatannejad, A., Tavilani, H., Sadeghi, M. R., Amanpour, S., Shapourizadeh, S., & Doosti, M. Evaluation of ROS-TAC score and DNA damage in fertile normozoospermic and infertile asthenozoospermic males. Urology journal 2017,14(1), 2973-2978.
9. Huang, C., Cao, X., Pang, D., Li, C., Luo, Q., Zou, Y., ... & Chen, Z.. Is male infertility associated with increased oxidative stress in seminal plasma? A-meta-analysis. Oncotarget 2018,9(36), 24494.
10. Riaz, M., Mahmood, Z., Shahid, M., Saeed, M. U. Q., Tahir, I. M., Shah, S. A., ... & El-Ghorab, A. Impact of reactive oxygen species on antioxidant capacity of male reproductive system. International journal of immunopathology and pharmacology 2016,29(3):421-425.
11. Issa Layali, E. T., Joulaei, M., Jorsaraei, S. G. A., & Farzanegi, P. Total antioxidant capacity and lipid peroxidation in semen of patient with hyper viscosity. Cell Journal 2015,16(4):554.
12. Roychoudhury, S., Sharma, R., Sikka, S., & Agarwal, ADiagnostic application of total antioxidant capacity in seminal plasma to assess oxidative stress in male factor infertility. Journal of assisted reproduction and genetics 2016,33(5):627-635.