The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 3402-3407

Seminal angiotensin II type 2 receptor (AT2R) as a new biomarker for idiopathic Oligo-asthenozoospermia

Ashraf Hassan Ahmed Hassan, AdelAbd El-Kader Zalata,
Mohamed Khaled Galal Selim, Mohamed Ibrahim Abdel-Wahab*
Department of Dermatology, Andrology and STDs, Faculty of Medicine, Mansoura University**,** Egypt
***Corresponding author:** Mohamed Ibrahim Abdel-Wahab, **Mobile:** (+20) 01062736853,
E-Mail: drmohamedsalamah1998@gmail.com

ABSTRACT

Background: Infertility is defined as failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse. Angiotensin II can bind to two different receptors : angiotensin receptor type 1 (AT1R) and type 2 (AT2R). There is evidence that the components of renin-angiotensin system (RAS) are locally synthesized in the male reproductive tissues as in the epididymis, prostate, testes, seminal fluid, and spermatozoa.

Objective: The aim of the present study is to determine the presence of soluble AT2R in human seminal plasma and to evaluate its possible role as a new diagnostic biomarker for cases of idiopathic oligo-asthenozoospermia.

Patients and methods: This study was conducted as a case-control study at Mansoura University Hospitals and included 100 subjects. Participants were divided into 50 patients with idiopathic oligo-asthenozoospermia, and 50 healthy volunteers who had achieved conception within one year of continuous unprotected marital relationship with normal semen analysis. All participants were subjected to thorough history taking, complete clinical examination, Doppler US for varicocele and semen analysis. The soluble angiotensin II type 2 receptors (AT2R) were measured in the seminal plasma by angiotesnin II type 2 receptor ELISA kit**.**

Results: Seminal AT2R, α-glucosidase and acrosin activity index were significantly decreased in idiopathic oligo-asthenozoospermia group in comparison with the control group. Seminal AT2R and α-glucosidase were negatively correlated with WBCs. The sperm concentration, grade A motility, grade (A+B) motility, velocity, linear velocity, linearity index, and normal morphology exhibited highly significant positive correlations with seminal plasma AT2R and alpha-glucosidase. Seminal AT2R is highly significant negatively correlated to malondialdehyde.

Conclusion: The present study revealed that AT2R is present in human seminal plasma and has a positive correlation with sperm concentration and motility. In addition, AT2R was significantly lower in idiopathic oligo-asthenozoospermia patients compared with healthy controls. The positive correlation of AT2R expression with motility highlights its potential role in idiopathic oligo-asthenozoospermia. These findings point to the possible involvement of AT2R in infertility.

Keywords: Male infertility, Idiopathic oligoasthenozoospermia, Renin angiotensin system, Angiotensin II type 2 receptor in asthenozoospermia.

INTRODUCTION

Idiopathic oligoasthenoteratozoospermia (iOAT) means defective spermatogenesis of unknown etiology and is regarded as undetectable by the common laboratory methods. iOAT can be classified as: isolated astheno± teratozoospermia (no alteration in sperm concentration); moderate (sperm concentration < 20 × a10^6/mL and > 5 ×10^6/mL); or severe (sperm concentration< 5 × 10^6/mL) (1).There is higher levels of angiotensin II type 2 receptors (AT2R) in the fetus and neonate. The AT2R is still controversial and enigmatic.ATR2 is hyposthesized to mediate some effects as inhibition of cell growth , formation of fetal tissue, the extracellular matrix modulation ,neuronal regeneration, apoptosis, cellular differentiation, and maybe vasodilation and left ventricular hypertrophy[(2)]. **.Ariny *et al.*** [(3)] investigated the effects of varicocelectomy on the levels of angiotensin II in sperm and the expression of the AT2R on spermatozoa in varicocele patients in relation to their reproductive status. They came to the conclusion that angiotensin II and AT2R dysregulation may occur in varicocele patients, which may contribute to varicocele-related infertility.

The aim of the present study is to determine the presence of soluble AT2R in human seminal plasma and to evaluate its possible role as a new diagnostic marker for cases of iOAT.

PATIENTS AND METHODS

A case-control study was carried out on subjects from Andrology Outpatient Clinic at Mansoura University Hospitals, for one year. The study included 100 subjects, who were divided into 2 groups:

1. Idiopathic oligo-asthenozoospermia (iOAT)group (n= 50).
2. Control Group (n= 50) included healthy volunteers who had achieved conception within one year of continuous unprotected marital relationship with normal semen analysis.

Inclusion criteria:

- Age from 20 to 50 years old.
- Control group of healthy age-matched males who have achieved pregnancy recently.
- All patients have oligo-asthenozoospermia.

Exclusion criteria:

- Patients with history of local diseases or systemic illnesses, medical treatmet, or surgeries that may

Received: 28/09/2022
Accepted: 01/12/2022

have a negative impact on fertility.

- Patients with varicocele diagnosed by clinical assessment and doppler ultrasonography.
- Patients who have hypogonadism or history of pelvic or spinal injuries.
- Patients with genital deformity.
- Azoospermia patients were not included in this study.

All participants went through:

- Thorough history taking.
- A general physical examination to determine any abnormalities in the endocrine, cardiovascular, pulmonary, gastro-intestinal, and neurological systems that may affect fertility.
- A local genital examination to rule out any possible abnormalities, including the testis, epididymis, vas deferens, and inguinal area.
- Color Doppler ultrasound grading of reflux.
- Semen analysis.

Techniques performed on human spermatozoa:

Computer-assisted semen analysis (auto sperm): In this work, sperm motility and concentration were assessed using simple computer -assisted (Autosperm) method [(4)]:

Assay of acrosin activity (by gelatin-covered microslides and gelatinolysis):

Gelatin-covered slides were prepared by spreading 20 µL of 5 % gelatin (Merck, Darmstadt, Germany) in distilled water on the slides. The slides were then air-dried, stored at 4 C° overnight and fixed and washed in phosphate-buffered saline [(5)]. Semen samples of 20 µL were diluted 1:10 in PBS containing 15.7mmol/L α-D-glucose. Semen samples were smeared on prepared slides and incubated in a moist chamber at 37 C° for 2 h. The halo diameter around any 10 spermatozoa shown to be representative of sperm present in the ejaculate was measured in phase contrast with an eyepiece micrometer. The halo formation rate was calculated per slide as the percentage of spermatozoa showing a halo. One hundred spermatozoa were evaluated. An acrosin activity index was calculated by multiplying the halo diameter by the halo formation rate [(6)].

Techniques performed on seminal plasma:

The supernatant (seminal plasma) was divided into 3 tubes and kept at -20°C till analysis:

1. in the first tube , angiotensin II type 2 receptor ELISA kit was used to detect the presence of soluble angiotensin II type 2 receptors (AT2R) in the seminal plasma (Abbexa, Cambridge, UK).
2. Seminal alpha-glucosidase activity was measured in the second tube to test epididymal function [(7)].
3. To assess lipid peroxidation, malondialdehyde (MDA)was measured in the third tube using a colorimetric method [(8)].

Assay of expression of angiotensin II type 2 receptor in seminal plasma by ELISA:

The level of soluble angiotensin II receptor 2 (AT2R) in the seminal plasma was estimated by angiotesnin II type 2 receptor ELISA kit (Abbexa, Cambridge, UK). This kit is used to assay the angiotensin II receptor 2 (AT2R) in the sample of human's serum, blood plasma, saliva, urine and other related tissue liquid.

Assay of alpha-glucosidase activity: The level of alpha glucosidase was measured using Episcreen Kit; FertiPro, 32,8730Lotenhulle, Belgium.

Determination of MDA 'thiobarbituric acid reactive substances' [(9)]:

Trichloroacetic acid (TCA) is added o precipitate the proteins of seminal plasma.). Then, thiobarbituric acid (TAB) reacts with MDA to form thiobarbituric acid reactive product which is measured at 534 nm.

Ethical Approval:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Mansoura University. Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

Statistical analysis was done by using MedCalc® program version 8.1. 2005**.** The results of this study were nonparametric data according to chi-squared test. The nonparametric data were expressed as median , and range . Mann-Whitney test was used as a test of significance for comparison of two groups. The qualitative data were presented in the form of number and percentage. Spearman rank correlation coefficient was done to study the relation between variables. Receiver-Operating Characteristic (ROC) was done to determine a cutoff point and sensitivity, specificity, and accuracy was done for this cut off point. comparison of roc curves were done for statistical significance among different variables.

RESULTS

Table (1) shows the semen parameters which include volume (ml), concentration (million/ml), grade A motility (%), grade A + grade B motility (%), velocity (µm/second), linear velocity (µm/second), linearity index (%), normal morphology (%) and WBCs (million/ml) in different groups. In comparison between control group and the idiopathic OA group, concentration, grade A motility, grade A + grade B motility, velocity, linear velocity, linearity index and normal morphology were significantly decreased in idiopathic OA group. As regard WBC count, it was significantly increased in idiopathic OA group than controls.

Table (1): Semen parameters of control group and idiopathic oligo-asthenozoospermia group.

Parameters	Control (n= 50)	iOAT (n= 50)	P-value
Volume (ml)	3.3 (1.7-5.8)	3.1 (1.7-5.1)	0.39 (NS)
Concentration (million/ml)	54.04 (31.2-79.1)	16.8 (5.3-19.8)	<0.001 (HS)
Grade A motility (%)	42.5 (33-52)	17.5 (3-30)	<0.001 (HS)
Grade A + grade B motility (%)	66 (48-82)	30.5 (9-44)	<0.001 (HS)
Velocity (µm/second)	42 (29-55)	27 (16-38)	<0.001 (HS)
Linear velocity (µm/second)	32.3 (24.2-41.9)	16 (4.2-23.4)	<0.001 (HS)
Linearity index (%)	78.9 (70.6-92.6)	59 (32.8-74)	<0.001 (HS)
Normal morphology (%)	42 (32-55)	15.9 (4.3-19.2)	<0.001 (HS)
WBCs (million/ml)	0.6 (0.1-0.9)	1.1 (0.6-1.8)	<0.001 (HS)

Data are expressed as median and range

Table(2) shows α-glucosidase (mu/ml), acrosin activity index (%), malondialdehyde (nmol) and AT2R (ng/ml) in all studied groups. α-glucosidase, acrosin activity index and AT2R were significantly decreased in iOAT group in comparison with controls. As regard seminal plasma malondialdehyde, it was found significantly higher in iOAT group than controls.

Table (2): Seminal plasma alpha-glucosidase, acrosin activity index, malondialdehyde (MDA) and AT2R of control group and iOAT group.

Parameters	Control (n= 50)	IOAT (n= 50)	P-value
α-glucosidase (mu/ml)	44.5 (23-68)	23 (12-34)	<0.001 (HS)
Acrosin activity index (%)	16 (6-26)	6.5 (2-10)	<0.001 (HS)
Malondialdehyde (MDA) (nmol/l)	26.4 (16.8-32.5)	34 (20.6-47.9)	<0.001 (HS)
AT_2R (ng/ml)	16.4 (10.5-22.4)	7.5 (2.9-14.5)	<0.001 (HS)

Data are expressed as median and range

Table (3) shows the correlation of seminal plasma AT2R and alpha-glucosidase with sperm parameters of all studied groups. Table (4) shows the correlation of MDA and acrosin activity index with sperm parameters of all studied groups.

Table (3): Correlation of seminal plasma AT2R and alpha-glucosidase with semen parameters in studied groups.

Variable	AT_2R		α-glucosidase	
	R	P-value	R	P-value
Concentration (million/ml)	0.7	<0.001 (HS)	0.6	<0.001 (HS)
Grade A motility (%)	0.6	<0.001 (HS)	0.7	<0.001 (HS)
Grade A + grade B motility (%)	0.6	<0.001 (HS)	0.7	<0.001 (HS)
Velocity (µm/second)	0.6	<0.001 (HS)	0.5	<0.001 (HS)
Linear velocity (µm/second)	0.7	<0.001 (HS)	0.6	<0.001 (HS)
Linearity index (%)	0.7	<0.001 (HS)	0.6	<0.001 (HS)
Normal morphology (%)	0.7	<0.001 (HS)	0.6	<0.001 (HS)
WBCs (million/ml)	-0.5	<0.001 (HS)	-0.5	<0.001 (HS)

Table (4): Correlation of MDA and acrosin activity index with semen parameters in studied groups.

Variable	Malondialdehyde (MDA)		Acrosin activity index	
	R	P-value	R	P-value
Concentration (million/ml)	-0.4	0.001 (HS)	0.7	<0.001 (HS)
Grade A motility (%)	-0.5	<0.001 (HS)	0.7	<0.001 (HS)
Grade A + grade B motility (%)	-0.5	<0.001 (HS)	0.7	<0.001 (HS)
Velocity (µm/second)	-0.2	0.01 (S)	0.6	<0.001 (HS)
Lienar velocity (µm/second)	-0.3	0.001 (HS)	0.61	<0.001 (HS)
Linearity index (%)	-0.4	<0.001 (HS)	0.7	<0.001 (HS)
Normal morphology (%)	-0.4	<0.001 (HS)	0.8	<0.001 (HS)
WBCs (million/ml)	0.3	<0.001 (HS)	-0.6	<0.001 (HS)

Table (5) shows correlation of seminal plasma AT2R with alpha-glucosidase, malondialdehyde and acrosin activity index of all studied groups. It was found that AT2R is highly significant negative correlated to malondialdehyde. On the other hand, there were highly significant positive correlations between AT2R and both of α- glucosidase and acrosin activity index.

Table (5): Correlation of seminal plasma AT2R with alpha glucosidase, MDA and acrosin activity index in studied groups

Variable	AT2R	
	R	**P-value**
α-glucosidase (mu/ml)	0.5	<0.001 (HS)
malondialdehyde (MDA) (nmol/l)	-0.4	<0.001 (HS)
Acrosin activity index (%)	0.6	<0.001 (HS)

Table (6) shows correlation of seminal plasma AT2R with hormonal profile of all studied groups. It was found that AT2R is significantly negatively correlated to prolactin

Table (6): Correlation of seminal plasma AT2R with hormonal profile (FSH, LH, testosterone, prolactin and estradiol).

Variable	AT2R	
	R	**P-value**
FSH (mIU/ml)	-0.04	0.7 (NS)
LH (mIu/ml)	0.04	0.7 (NS)
Testosterone (ng/ml)	-0.16	0.11 (NS)
Prolactin (ng/ml)	-0.28	0.01 (S)
E_2 (pg/ml)	0.015	0.88 (NS)

Table (7) found that AT_2R at a criterion value (10.3) can distinguish control group from idiopathic OA patients with specificity of 100%, sensitivity of 72% and area under the curve of 0.9. Also, acrosin activity index at a criterion value (9) can distinguish control group from idiopathic OA patients with specificity of 88%, sensitivity of 98%, and area under the curve of 0.97. Alpha-glucosidase at a criterion value (34) can distinguish control group from idiopathic OA patients with specificity of 68%, sensitivity of 100% and area under the curve of 0.92. As regards malondialdehyde, it was found that it can discriminate between control and idiopathic OA groups at a criterion value (32.5) with specificity of 100%, sensitivity of 54% and area under the curve of 0.8.

Table (7): Criterion values and coordinates of ROC curves of AT2R.

Variables	Criterion value	Sensitivity	Specificity	Area Under Curve
AT2R	≤10.3	72	100	0.9
Acrosin activity index	≤9	98	88	0.97
Alpha-glucosidase	≤34	100	68	0.92
Malondi-aldehyde (MDA)	>32.5	54	100	0.8

DISCUSSION

Good sperm motility, which is crucial for male fertility, is considered the most reliable predictor of male infertility. Fertilization rates, embryo quality, and pregnancy rates are poorer when spermatozoa with low motility have been used for assisted reproductive techniques as compared to those with normal motility. Yet, a better understanding of the molecular mechanisms underlying the formation of motile sperm would enable us to emphasize the problem of diminished motility and sperm infertility [(10)].

The renin-angiotensin system (RAS) is implicated to have a regulatory role in reproduction. It has been suggested that a testis-specific isoform of ACE is involved in regulation of sperm function and capacitation, via affecting angiotensin I and II, enkephalins, and bradykinin, ACE can indirectly affect fertility. Angiotensin I is changed into angiotensin II by ACE, and angiotensin II is known to stimulate sperm motility [(11)].

The RAS active peptide, angiotensin II, regulates a variety of biological functions, including reproduction. Angiotensin type 1 receptors (AT1R) and angiotensin type 2 receptor (AT2R) mediate the actions of Ang II. Capacitation and the acrosome response are also related to AT1R. Angiotensin receptor type 2 is localized in the epididymis, testes, prostate, and mouse spermatozoa[(12)].

It was found that the effects of AT2R are opposite to those mediated by AT1R. AT2R was found in the epididymis and on the acrosomal portion of the sperm head in the male reproductive system. These results support the idea that RAS may play a regulatory role in reproduction [(13)].

Previously, AT2R was found by immunocytochemistry on the mouse acrosomal area, where it was thought to be involved in the acrosome reaction. Nevertheless, its action was believed to be independent of calcium-mediated signals. AT2R was also found on the human sperm head's equatorial and post-acrosomal regions, indicating its role in sperm processes crucial for reproduction [(14)].

This study showed that in comparison between control group and the idiopathic OA group, concentration, grade A motility, grade A + grade B motility, velocity, linear velocity, linearity index and normal morphology were significantly decreased in idiopathic OA group. However, volume didn't show significant decrease in idiopathic OA group than controls. As regard WBC count, it was significantly increased in idiopathic OA group than controls. In harmony with the results of our study**, El-Ariny *et al.*[(3)]** studied the levels of angiotensin II in semen and angiotensin II type 2 receptor expression on spermatozoa in varicocele patients in relation to their fertility status. Sperm concentration, normal morphology and progressive motility were significantly lower in infertile varicocele patients (pre-operative) compared with the control and fertile varicocele groups. They were also significantly lower in fertile varicocele patients compared with the control group. The post-operative values for sperm concentration and progressive motility in the infertile varicocele group were significantly lower than in the fertile varicocele and the control groups, meanwhile, the post-operative normal sperm morphology % was significantly lower in the infertile varicocele group compared with the control group but not the fertile varicocele group.

The results of this study demonstrated that seminal AT_2R, α-glucosidase and acrosin activity index were significantly decreased in idiopathic OA group in comparison with controls. As regard seminal plasma malondialdehyde, it was found significantly higher in idiopathic OA group than controls. **Gianzo *et al.*[(14)]** determined the presence and distribution of AT2R in human spermatozoa and its implication regarding fertility status and found that the percent of AT2R positive spermatozoon was significantly higher in normozoospermic compared with azoospermic males.

In this study, the levels of FSH and LH were found to be non-significantly increased in control group than in idiopathic OA group. However, non-significant increase in testosterone, prolactin and E_2 in idiopathic OA group than control group was found. **El-Ariny *et al.*[(3)]** detected no significant difference among the studied groups regarding age, FSH, LH or serum testosterone levels.

The results of this study showed that seminal AT2R and alpha-glucosidase were negatively correlated with WBCs. However, they showed highly significant positive correlations with sperm concentration, grade A motility, grade (A+B) motility, velocity, linear velocity, linearity index and normal morphology.

Gianzo *et al.*[(14)] detected a significant positive correlation between AT2R expression and sperm concentration but not with morphology. This difference as regards sperm concentration could be attributed to the heterogeneous nature of their study population as regards fertility status. Regarding sperm motility, the percentage of PR spermatozoa was significantly and positively correlated with the percentage of AT2R-positive spermatozoa in fresh samples. Accordingly, they observed a significant negative correlation between the percentage of AT2R-positive cells and IM spermatozoa in these samples. They stated that angiotensin II type 2 receptor is present in human semen and may be involved as a biomarker of sperm motility.

CONCLUSION

AT2R is present in human seminal plasma and has a positive correlation with sperm concentration and motility. In addition, AT2R was significantly lower in idiopathic oligo-asthenozoospermia patients compared with healthy controls. The positive correlation of AT2R expression with motility highlights its potential role in idiopathic oligo-asthenozoospermia. These findings point to the possible involvement of AT2R in infertility.

AT2R could be involved in the control of sperm motility, and it could be considered as a potential marker of sperm motility and as a potential tool to predict male fertility status

Supporting and sponsoring financially: Nil.
Competing interests: Nil.

REFERENCES

1. **Bonanomi N, Lucente G, Silvestrini B (2002):** Male fertility: core chemical structure in pharmacological research. Contraception, 65:317-20.
2. **D'Amore A, Black M, Thomas W (2005):** The angiotensin II type 2 receptor causes constitutive growth of cardiomyocytes and does not antagonize angiotensin II type 1 receptor-mediated hypertrophy. Hypertension, 46 (6):1347-54.
3. **El-Ariny A, El-Garem Y, Eid A *et al.* (2020):** Seminal levels of angiotensin II and angiotensin II type 2 receptor expression on spermatozoa in varicocele patients: Relation to fertility status. Andrologia, 20:e13513. Doi: 10.1111/and.13513
4. **Hinting A, Comhaire F, Schoonjans F (1988):** Capacity of objectively assessed sperm motility characteristics in differentiating between semen of fertile and subfertile men. Fertility and Sterility, 50(4):635-9.
5. **Henkel R, Müller C, Miska W *et al.* (1995):** Acrosin activity of human spermatozoa by means of a simple gelatinolytic technique: a method useful for IVF. Journal of Andrology, 16(3):272-7.
6. **Zalata A, Ahmed A, Allamaneni S *et al.* (2004):** Relationship between acrosin activity of human

spermatozoa and oxidative stress. Asian J Androl., 6(4):313-8**.**

7. **Guerin J, Ben Ali H, Rollet J *et al.* (1986):** α-Glucosidase as a Specific Epididymal Enzyme Marker Its Validity for the Etiologic Diagnosis of Azoospermia. Journal of Andrology, 7(3):156-62.
8. **Draper H, Squires E, Mahmoodi H *et al.* (1993):** A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radical Biology and Medicine, 15(4):353-63.
9. **Walker P, Shah S (1988):** Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. The Journal of Clinical Investigation, 81(2):334-41.
10. **Bhilawadikar R, Zaveri K, Mukadam L *et al.* (2013):** Levels of Tektin 2 and CatSper 2 in normozoospermic and oligoasthenozoospermic men and its association with motility, fertilization rate, embryo quality and pregnancy rate. J Assist Reprod Genet., 30:513-23.
11. **Ojaghi M, Kastelic J, Thundathil J (2017):** Testis-specific isoform of angiotensin-converting enzyme (tACE) is involved in the regulation of bovine sperm capacitation. Molecular Reproduction and Development, 84(5):376-88.
12. **Skov J, Persson F, Frokiaer J *et al.* (2014):** Tissue Renin-Angiotensin systems: a unifying hypothesis of metabolic disease. Front Endocrinol (Lausanne), 5:23. Doi: 10.3389/fendo.2014.00023
13. **Singh K, Karnik S (2016):** Angiotensin receptors: Structure, function, signaling and clinical applications. Journal of Cell Signaling, 1(2):111. Doi: 10.4172/jcs.1000111
14. **Gianzo M, Munoa-Hoyos I, Urizar-Arenaza I *et al.* (2016):** Angiotensin II type 2 receptor is expressed in human sperm cells and is involved in sperm motility. Fertil Steril., 105:608-16.