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男性精浆中 IL-6 和 sICAM-1 与免疫性不育的关系研究

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摘要 目的:探究男性精浆中白细胞介素-6(IL-6)和可溶性细胞黏附分子-1(sICAM-1)与免疫性不育的关系。方法:选择2014年6月至2015年12月我院收治不育症患者189例及100例健康体检者为研究对象,根据患者精子混合抗球蛋白反应实验(MAR)结果将不育症患者分为免疫性不育组(88例)和非免疫性不育组(101例);免疫不育组患者按照精液白细胞过氧化物酶染色情况分为免疫性阳性白细胞组(WBC $\geq 1 \times 10^6/\text{mL}$)36例和免疫性阴性白细胞组($<1 \times 10^6/\text{mL}$)52例,分析并比较各组间精子质量、IL-6和sICAM-1水平。结果:免疫性不育组与非免疫性不育组患者精液精子向前运动比率、存活率均低于对照组($P<0.05$);免疫性不育组与非免疫性不育组精液各项参数均不存在差异($P>0.05$);免疫性不育组患者IL-6、sICAM-1水平均高于非免疫性不育组及对照组,差异有统计学意义($P<0.05$);非免疫性不育组IL-6、sICAM-1水平均高于对照组,差异有统计学意义($P<0.05$)。相关性分析显示,精子被IgG黏附数比例与研究对象精液IL-6、sICAM-1水平呈正相关关系($r=0.438, 0.561; P<0.05$);免疫性阳性白细胞组患者精液IL-6与sICAM-1水平均高于免疫性阴性白细胞组($P<0.05$)。结论:免疫性不育症患者精子质量下降,男性精液中IL-6和sICAM-1水平表达越高, MAR阳性率越高,男性免疫性不育发生的可能性越大。

关键词: 免疫性不育; IL-6; sICAM-1

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Study on the Relationship between the Level of IL-6, sICAM-1 and Immune Infertility in Male Seminal Plasma

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ABSTRACT Objective: To explore the relationship between the level of IL-6, sICAM-1 and immune infertility in male seminal plasma. **Methods:** 189 cases of infertile patients and 100 cases of healthy physical examination people were selected as the research objects from June 2014 to December 2015, and the infertility patients were divided into immune infertility group (88 cases) and non-immune infertility group (101 cases) according to the results of patient's sperm with Mixed Agglutination Reaction (MAR); Patients in immune infertility group were divided into immune positive cells group (WBC is more than or equal to $1 \times 10^6/\text{mL}$) (36 cases) and immune negative leukocyte negative group ($<1 \times 10^6/\text{mL}$) of 52 cases accordance to the seminal leucocyte peroxidase staining; To analysis and compare the sperm quality, the level of IL-6 and sICAM-1 between the each groups. **Results:** The rate of sperm forward movement and the survival rate of the sperm in immune infertility group and non-immune infertility group were lower than those of the control group ($P<0.05$); There were no differences in semen parameters between the immune infertility group and the non-immune infertility group ($P>0.05$); The levels of IL-6 and sICAM-1 in the immune infertility group were significant higher than those in the non-immune infertility group and the control group ($P<0.05$); The levels of IL-6 and sICAM-1 in the non-immune infertility group were significant higher than those in control group ($P<0.05$); Correlation analysis showed that the sperm were IgG adhesion ratio were positively correlated with the level of IL-6, sICAM-1($r=0.438, 0.561, P<0.05$) among research object; The levels of IL-6 and sICAM-1 in the patients with immune positive leukocyte were higher than those of the immune negative leukocyte group ($P<0.05$). **Conclusion:** The sperm quality of immune infertility patients were decreased, the higher expression of IL-6 and sICAM-1 in male semen, the higher MAR positive rate, the greater the possibility of male immune infertility.

Key words: Immune infertility; IL-6; sICAM-1

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前言

随着社会发展及人们生活的不断变化,近年来不育症的发生率呈现逐年上升趋势^[1]。据调查^[2],全球不育症患病率约5%~30%不等,其中约40%由男性方面因素导致不孕不育。关于不孕症的发病因素较多,研究报道约2%~10%的不育症与免疫因素有关,被称为免疫性不育^[3],其定义为以男性精子为抗原,在体内与抗体发生免疫反应导致不育症,也是WHO对不育症分类的因素之一。既往研究报道^[4],细胞因子在男性生殖系统的病理变化及免疫调节作用会对男性睾丸功能及精子发育产生一定影响。白细胞介素-6(interleukin-6, IL-6)是细胞因子中最核心的成员,介导机体的炎症免疫反应,而可溶性细胞黏附分子-1(soluble inter cellular adhesion molecule-1, sICAM-1)能够诱导单核巨噬细胞在机体病变处浸润,释放炎症因子进一步加重炎症浸润。因此,本研究从炎症介导角度探究精液中炎症标志物sICAM-1、IL-6在预测免疫性不育发生的作用,探讨炎症细胞因子在男性免疫性不育发病过程中的作用,现报告如下。

1 对象与方法

1.1 研究对象

选择2014年6月至2015年12月我院收治不育症患者男性为研究对象。纳入排除标准:(1)所有患者均被检查患有不育症,诊断标准采用2000年WHO提出男性不育的诊断标准^[5];(2)所有患者夫妻性生活正常,均未采取避孕措施;(3)女方均进行妇科检查、B超检查、内分泌及输卵管检查等排除女方不孕情况;(4)排除合并遗传、血液系统疾病、慢性阻塞性肺病、内分泌、免疫系统疾病、心肝肾功能不全及合并恶性肿瘤患者;(5)所有患者及家属均知情同意,本研究所有操作均符合医学伦理学道德,并经院伦理委员会审核通过。经纳入排除标准共收集不育患者189例,根据患者精子混合抗球蛋白反应实验(Mixed antiglobulin reaction, MAR)的结果将不育症患者分为免疫性不育组(精子被黏附数 $\geq 50\%$)88例和非免疫性不育组(精子被黏附数 $<50\%$)101例。免疫性不育组患者按照精液白细胞过氧化物酶染色情况分为免疫性阳性白细胞组(WBC $\geq 1 \times 10^6/\text{mL}$)36例和免疫性阴性白细胞组($<1 \times 10^6/\text{mL}$)52例,免疫性不育组患者年龄24~39岁,平均年龄(28.97 $\pm$ 8.58)

岁;婚龄2~14年,平均婚龄(5.22 $\pm$ 3.23)年;病程1~9年,平均病程(3.64 $\pm$ 3.12)年;非免疫性不育组患者年龄23~37岁,平均年龄(28.63 $\pm$ 7.36)岁;婚龄3~16年,平均婚龄(4.89 $\pm$ 3.35)年;病程2~9年,平均病程(3.01 $\pm$ 2.89)年;另选择同期我院健康体检男性100例为对照组,其年龄25~38岁,平均年龄(28.67 $\pm$ 7.38)岁;婚龄2~15年,平均婚龄(5.11 $\pm$ 3.42)年,三组间年龄、婚龄均无差异($F=1.231, 0.783; P>0.05$)。

1.2 研究方法

1.2.1 收集标本 根据WHO标准,采用手淫的方式收集精液。所有研究要求禁欲2~7天,排尿后,用肥皂清洗双手和阴茎,冲洗掉肥皂沫后,用新的一次性毛巾擦干双手和阴茎后进行精液收集。

1.2.2 观察指标 依据第五版WHO颁布《人类精液检查与处理实验室手册》对收集精液进行分析^[6]。在显微镜的辅助下采用肉眼辨别判读精液的液化时间,采用计算机辅助精子分析系统(计算机辅助精子分析(computer assisted sperm analysis, CASA),所用仪器为Hamilton Thorne CASA,)对患者精子浓度与活力进行测量;采用精子混合抗球蛋白反应实验(mixed antiglobulin reaction, MAR)检测精子膜表面抗体,试剂购于深圳华康生物医学工程有限公司;采用伊红染色法检测精子存活率;采用正甲苯胺法检测免疫性不育组患者白细胞过氧化物染色情况,试剂购于深圳华康生物医学工程有限公司;采用酶联免疫吸附试验(ELISA)测定患者精液IL-6、sICAM-1水平,试剂盒购于美国Bender Med Systems公司(批号:D130301、D130219),本研究所有操作均严格按照试剂盒说明书进行。

1.3 统计学分析

采用SPSS 19.0对收集资料进行统计学分析,两组间定量资料均数比较采用独立样本t检验,多组定量资料间均值比较采用F检验,组内两两比较采用SNK法,相关性分析采用Pearson相关,以 $P<0.05$ 表示差异有统计学意义。

2 结果

2.1 各组间精液各项常规参数比较

免疫性不育组与非免疫性不育组患者精液精子向前运动比率、存活率均低于对照组($P<0.05$),精液液化时间、精子浓度与对照组无差别($P>0.05$);免疫性不育组与非免疫性不育组精液各项参数均不存在差异($P>0.05$),见表1。

表1 各组间精液各项常规参数比较

Table 1 Comparison of the normal parameters of semen among the groups

Groups	N	Semen liquefaction time(min)	Sperm viability(%)	Sperm conc($\times 10^6$ /mL)	Forward movement sperm percentage(%)
Immune infertility group	88	30.12 $\pm$ 13.23	47.97 $\pm$ 14.34*	90.99 $\pm$ 40.34	27.53 $\pm$ 6.12*
Non Immune infertility group	101	29.78 $\pm$ 12.84	50.34 $\pm$ 17.81*	94.13 $\pm$ 38.03	30.01 $\pm$ 7.93*
Control group	100	28.29 $\pm$ 13.99	70.11 $\pm$ 12.33	104.32 $\pm$ 59.31	53.23 $\pm$ 8.38
F		1.893	17.345	2.543	18.304
P		0.781	<0.001	0.351	<0.001

Note: Compared with control group, * $P<0.05$.

2.2 各组间精液中IL-6、sICAM-1水平比较

免疫性不育组患者IL-6、sICAM-1水平均高于非免疫性不育组及对照组,差异有统计学意义($P<0.05$);非免疫性不育组

IL-6、sICAM-1水平均高于对照组,差异有统计学意义($P<0.05$),见表2。

表 2 各组间精液中细胞因子水平比较

Table 2 Comparison of the levels of cytokines in seminal fluid between groups

Groups	N	IL-6(ng/L)	sICAM-1 (ng/mL)
Immune infertility group	88	38.01± 15.32**	88.93± 32.12**
Non Immune infertility group	101	22.19± 11.93*	57.93± 21.93*
Control group	100	8.91± 3.93	18.76± 7.88
F		31.232	40.836
P		<0.001	<0.001

Note: Compared with control group, *P<0.05; Compared with Non Immune infertility, #P<0.05.

2.3 免疫性阳性白细胞组和免疫性阴性白细胞组比较

于免疫性阴性白细胞组(P<0.05),见表 3。

免疫性阳性白细胞组患者精液 IL-6 与 sICAM-1 水平均高

表 3 免疫性阳性白细胞组和免疫性阴性白细胞组比较

Table 3 Comparison of the white blood cell group and the white blood cell group

Groups	N	IL-6(ng/L)	sICAM-1 (ng/mL)
Leukocyte-positive group	36	47.18± 18.34	101.23± 36.43
Leukocyte-negative group	52	31.77± 15.34	79.89± 22.74
t		-4.278	-3.382
P		<0.001	<0.001

2.4 精子被 IgG 或 IgA 黏附数比例与 IL-6、sICAM-1 水平相关性分析

相关性分析显示,精子被 IgG 或 IgA 黏附数比例与研究对象精液 IL-6、sICAM-1 水平呈正相关关系($r=0.438, 0.561; P<0.05$)。

3 讨论

调查显示^[8],近年来不育症的发生率呈现逐年上升趋势,全球不育症的患病率约 5%~30%。研究显示^[9],导致不育症的发病因素较多,其中,免疫因素是重要的因素之一,也是 WHO 定义不育症分类的一个标准。2000 年 WHO 将免疫性不育症定义为^[10]:男性射精及性功能正常,每份精液中在 IBT 中至少有 50% 的活动精子被抗体覆盖。大部分研究者认为^[11],血-睾屏障出现调节异常,使精子细胞、精母细胞、精子与免疫系统发生免疫反应,生成抗精子抗体,因而导致免疫性不育的发生。目前,免疫性不育症的诊断方式主要有免疫珠试验、混合抗球蛋白反应试验及酶联免疫吸附法测体液抗精子抗体等方式^[12]。研究报道^[13],生殖系统中的相关隐性、慢性炎症反应与男性免疫性不育存在密切关系,机体内细胞因子介导的炎症反应在免疫性不育发病过程中存在重要作用。因此,本研究从炎症介导角度探究精浆中炎症标记物 sICAM-1 与 IL-6 在预测免疫性不育发生的作用。

研究报道^[14],精液的常规参数能够反应精子质量,本研究显示,免疫性不育组与非免疫性不育组患者精液精子向前运动比率、存活率均低于对照组(P<0.05),说明不育症患者精子质量均下降,但免疫性不育组与非免疫性不育组患者各项参数均不存在差异,说明精子质量在免疫性不育症的鉴别诊断上意义不明显,与既往研究结果一致^[15]。

细胞因子在睾丸不同细胞间信号调节与传导过程中其重要作用,其主要通过内分泌、旁分泌与自分泌形式调节机体内免疫状态、参与机体炎症反应对精子的成熟及功能产生影响。

研究报道^[16],单纯疱疹病毒、支原体、衣原体等生殖道感染导致男性免疫性不育的重要原因,因此本研究认为生殖系统中隐性、慢性炎症反应是导致男性免疫性不育的主要原因,而 IL-6 由机体巨噬细胞、单核细胞、血管内皮细胞及活化 T 细胞等产生,是细胞因子中最核心的成员,其介导机体的炎症免疫反应^[17];sICAM-1 主要表达于活化内皮细胞,是活化内皮细胞的标志,其能够诱导单核巨噬细胞在机体病变处浸润,释放炎症因子,如血管内皮生长因子(vascular endothelial growth factor, VEGF)、肿瘤坏死因子- α (Tumor necrosis factor- α , TNF- α)等,进一步加重炎症浸润^[18]。为此本研究探究炎症细胞因子在男性免疫性不育患者精浆的表达水平显示,免疫性不育组患者 IL-6、sICAM-1 水平均高于非免疫性不育组及对照组(P<0.05);非免疫性不育组 IL-6、sICAM-1 水平均高于对照组(P<0.05),说明精液中 IL-6 与 sICAM-1 水平与不育症存在关系,尤其是免疫性不育症。相关性分析显示,精子被 IgG 或 IgA 黏附数比例与研究对象精液 IL-6、sICAM-1 水平呈正相关关系($r=0.438, 0.561; P<0.05$),说明精液中 IL-6、sICAM-1 水平越高,精子被 IgG 或 IgA 黏附数比例越高,发生免疫性不育的发生率越大,与既往研究结果一致^[19,20]。此外,本研究显示免疫性阳性白细胞组患者精液 IL-6 与 sICAM-1 水平均高于免疫性阴性白细胞组(P<0.05),说明机体内部组织的炎症状态与精液中 IL-6 与 sICAM-1 表达可能存在一定关联。

综上所述,免疫性不育症患者精子质量下降,男性精液中 IL-6 和 sICAM-1 水平表达越高,MAR 阳性率越高,男性免疫性不育发生的可能性越大。

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