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Full Length Research Paper

The relationship between IL-17 and male infertility: Semen analysis

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The relationship between IL-17 and male infertility was investigated. Seminal fluid from 57 males, including infertile and normal, were selected and subjected to routine analysis and determination of the levels of cytokines. IL-17 can be detected in seminal fluid, with concentrations from 0.45 to 16.67 pg/ml. Semen analysis showed that semen samples with low activity sperms have higher IL-17 level, while IL-17 levels have no significant influence on sperm densities, morphologies and pH values. Sperm mortality rate of low IL-17 level samples is remarkably lower than that of high IL-17 level samples. Correlation analysis indicates that seminal fluid IL-17 and IL-6, IL-8, TNF-α were positively correlated, and seminal IL-17 level has no effects on bacteria colony counts and serum FSH, LH, T levels. Results indicate that IL-17 may play important roles in male reproduction.

Key words: Cytokine, IL-17, IL-6, IL-8, TNF-α.

INTRODUCTION

Cytokines are small peptides with different immune activities and secreted by a variety of cells. They bind to receptors of target cells and stimulate signal transduction, thereby regulating cell growth, proliferation, differentiation, and other functions. The biological role of cytokines is very extensive. Under normal circumstances, cytokines produced by testis immune cells, interstitial cells, sertoli cells, and spermatogonia cells function as intracellular signals that regulate growth and differentiation of germ cells, reproductive neuroendocrine, testicular function, and spermatogenesis. However, these regulations are mutual, and that various cells within the reproductive system can not only produce their own cytokines, but regulate the secretion of cytokines. If cytokine production is impaired, the reproductive system functions may be damaged, which leads to male infertility (Dousset et al., 1997).

IL-17 is a recently discovered pro-inflammatory cytokine, which is mainly secreted by the activated memory CD4 T cells. IL-17 is a glycoprotein with 155 amino acid residues and an N-terminal signal peptide. Binding to specific receptors, it promotes inflammation, immune responses, hematopoiesis, and other functions (Fossiez et al., 1996). It has been characterized having a strong inflammatory inducing activity, which promotes local production of chemokines such as IL-8, monocyte chemoattractant protein-1, and growth regulatory factor-α, thus inducing the rapid increase of monocytes and neutrophils. It stimulates the production of IL-6 and PGE-2, enhancing local inflammation. Studies showed that IL-17 is related to a variety of diseases, such as airway inflammation, tumor growth, and other chronic inflammatory diseases (systemic sclerosis, psoriasis scales, systemic lupus erythematosus, etc.) (Qin and Liu, 2003).

Although IL-17 level in semen was determined by Politch et al. (2007), the relationship between IL-17 level and seminal quality is still unknown. In this study, in order to study the roles of IL-17 in male infertility, we determined the IL-17 level in seminal fluid from both normal and infertile males, which were provided by Lianyungang Maternal and Child Hospital.

MATERIALS AND METHODS

Sampling

Semen samples were collected from 57 male patients of

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Table 1. The relationship between semen quality and IL-17, IL-6, IL-8, and TNF-α[a].

Parameter of semen	Number of samples	L-17 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
The percentage of sperm forward movement (a+b)					
< 50%	23	6.00±3.97	41.5±19.8	1049.5±1224.6	7.17±6.87
≧ 50%	34	3.37±2.09*	24.2±17.3*	645.6±539.5*	5.94±6.22**
Semen density					
< 60×10^6 /ml	22	4.60±2.17	36.3±17.1	788.9±950.0	6.79±5.01
≧ 60×10^6 /ml	35	4.25±3.73**	27.4±21.2**	809.7±851.3**	6.18±7.27**
The percentage of morphological normal sperm					
< 30%	41	4.75±3.46	32.4±20.1	788.4±752.1	6.16±6.53
≧ 30%	16	3.25±2.14**	24.1±17.7**	590.6±533.4**	7.59±6.66**
pH					
< 7.5	49	4.55±3.19	33.2±21.0	835.5±727.6	6.54.±6.73
≧ 7.5	8	3.35±3.29**	23.6±16.8**	563.7±512.2**	7.03±6.89**

[a](mean±SD) from 57 samples,* compared with control group, P<0.05,** compared with control group, P<0.01.

Lianyungang Maternal and Child Hospital, who had been abstinent for 3 to 5 days before the collection, from March to September, 2010. The average age of the patients, who received no antibiotic treatment, is 28.1 years. Semen samples were collected into sterile beakers in 37-degree water bath for liquefaction.

Semen routine analysis

After complete liquefaction, semen volume, pH, liquefaction time, appearance, density, and motility were analyzed according to the methods described by World Health Organization (1992). Sperm viability was also analyzed at 4, 8, and 24 h after sample collection.

Morphology assay

After liquefaction, semen samples were dried on coverslips and stained by modified Papanicolaou stain method (Gupta et al., 2010). Morphologies of at least 200 sperms of each sample were observed under microscope. The percentage of morphological normal sperm, abnormal head, abnormal neck and middle, abnormal tail, and cytoplasmic droplets were analyzed as described before (World Health Organization, 1992).

Calculation of bacteria colony

Semen fluids were diluted with sterile distilled water at 1:10, 1:100, or 1:1000, and 1 ml of each diluted sample was cultured in 10% sheep blood agar pre-warmed to 56°C. Numbers of colonies on each plate were counted 48 h after incubation at 37°C.

Seminal levels of IL-17, IL-6, IL-8, and TNF-α

After complete liquefaction, a fraction of each semen sample was spun at 600 g for 20 min. The supernatants were kept at -20°C before the levels of IL-17, IL-6, IL-8, and TNF-α were analyzed according to the method described by Kria et al. (1998).

Blood serum T, FSH, LH measurements

Blood serum T, FSH, and LH were measured by radioimmunoassay and the radioimmunoassay kits (DPC, USA) were used: for either FSH or LH determination, the dual antibody ^{125}I labeled reagent kits were used; as to T determination, the solid phase ^{125}I labeled reagent kit was used. The assays were performed according to the instructions of the radioimmunoassay kit.

Statistical analysis

Statistics was analyzed with SPSS 11.5 software and all data were shown as mean ± SD. Groups of data were compared with independent samples t-test and correlation was analyzed using linear regression.

RESULTS

The presence of IL-17 in seminal fluid

IL-17 was detected from all seminal fluid samples, with a concentration range from 0.45 to 16.67 pg/ml. The average level is 4.39±3.20 pg/ml.

Relationships of IL-17 level and semen quality

Semen samples are divided into two groups according to the percentage of sperm forward movement (a+b): low motility group ((a+b) < 50%) and high motility group((a+b) ≥50%). As shown in Table 1, IL-17 level of low motility group (6.00±3.97 pg/ml) is significantly higher

Table 2. The relationship between IL-17 concentration and the percentage of dead sperm in 4h, 8h, and 24h[a].

IL-17	Number of samples	Sperm mortality (%)		
		4 h**	8 h	24 h
< 4 pg/ml	23	13.3±11.2	36.1±15.6	63.5±15.5
≥4 pg/ml	34	24.7±19.2*	47.4±22.8*	75.5±18.6*
Total	57	17.7±15.6	40.5±19.3	68.2±17.6

[a](mean±SD) from 57 samples, * compared with control group, P<0.05, ** compared with control group, P<0.01.

Table 3. The relationship between IL-17 concentrations and outcome of bacterial count[a].

Colony count	Number of samples	IL-17(pg/ml)
< 10^4 CFU/ml	31	3.41±2.95
≥10^4 CFU/ml	8	3.66±2.07*
Total	39	3.59±2.72

[a](mean±SD) from39 samples, * compared with control group, P<0.05, ** compared with control group, P<0.01.

than that of high motility group (3.37±2.09 pg/ml) (P<0.01). The level of IL-17 is not relevant to sperm density, morphology, and pH of semen samples.

IL-17 level and sperm viability

Sperm viabilities of 37 semen samples were analyzed at 4, 8, and 24 h after collection. Table 2 shows that sperm mortality rates of low IL-17 level samples (< 4 pg/ml) are remarkably lower than those of high IL-17 level samples (≥4 pg/ml) (P < 0.05).

Semen fluid bacteria colony count and IL-17 level

Table 3 shows that IL-17 level does not affect the colony numbers of semen samples.

IL-17 level and levels of IL-6, IL-8 and TNF-α in semen fluid

Based on 57 semen samples (Table 4), both IL-6 and Il-8 concentrations are significantly lower in low IL-17 samples than those in high IL-17 samples (P<0.01), while TNF-α concentration remains same (P>0.05). Correlation analysis, as shown in Table 4, Figures 1, 2 and 3, indicates that seminal fluid IL-17 and IL-6, IL-8, TNF-α were positively correlated: correlation coefficient of IL-17 and IL-6 R^2 = 0.8941 (P <0.01); correlation coefficient of IL-17 and IL -8 R^2 = 0.8024, (P <0.01); correlation coefficient of IL-17 and TNF-α R^2 = 0.7145 (P <0.01).

Seminal fluid IL-17 level and plasma FSH, LH, T concentrations

We also measured the levels of serum reproductive hormones FSH, LH, and T of those 57 patients. As shown in Table 5, serum FSH, LH, and T levels have no significant differences between low IL-17 and high IL-17 groups.

DISCUSSION

IL-17 and semen quality

Earlier studies have shown that seminal fluid contains IL-1, IL-2, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, TNF-α, IFN-γ and other cytokines (Fossiez et al., 1996; Politch et al., 2007; Takaya et al., 2002). These cytokines play direct and indirect biological roles in sperm functions and spermatogenesis, which affect male fertility. There have been abundant reports on the effects of seminal IL-1, IL-2, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, TNF-α, and IFN-γ on sperm nature (Fossiez et al., 1996; Politch et al., 2007; Takaya et al., 2002), and we also found that IL-6 and IL-8 affect sperm motility, but do not affect density and morphology, while TNF-α has no effect on sperm characteristics. Here we report the impacts of IL-17 concentration on semen for the first time. We measured IL-17 level of semen from 57 male patients (both normal

Table 4. The concentration relationship between IL-17 and IL-6, IL-8, and TNF-α[a].

IL-17	Number of samples	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
< 4 pg/ml	28	15.71±9.19	374.4±216.2	5.09±4.65
≥ 4 pg/ml	29	45.47±16.52*	1214.2±1073.8*	7.70±7.67
Total	57	30.85±20.06	801.7±882.3	6.42±6.45

[a](mean±SD) from 57 samples, * compared with control group, P<0.05, ** compared with control group, P<0.01.

Table 5. The relationship between IL-17 concentrations in seminal plasma and the levels of FSH, LH, and T in serum[a].

IL-17	Number of samples	FSH (IU/L)	LH (IU/L)	T (nmol/L)
< 4 pg/ml	28	4.30±2.14	3.86±1.36	16.73±5.33
≥ 4 pg/ml	29	6.43±4.18	4.23±1.64	15.44±4.53
Total	57	5.08±3.15	3.99±1.45	16.26±5.02

[a](mean±SD) from 33 (normal group) and 24 (abnormal group),* compared with control group, P<0.05, ** compared with control group, P<0.01.

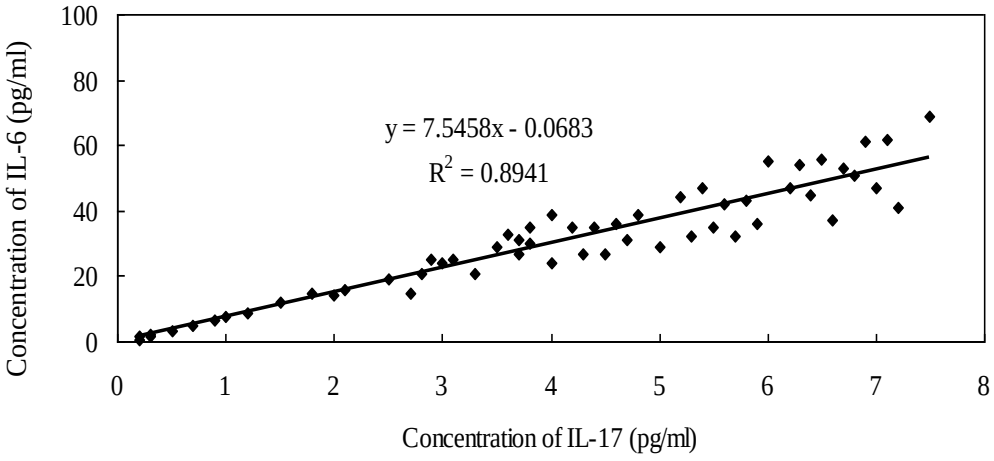

Figure 1. The correlation coefficient of IL-17 and IL-6.

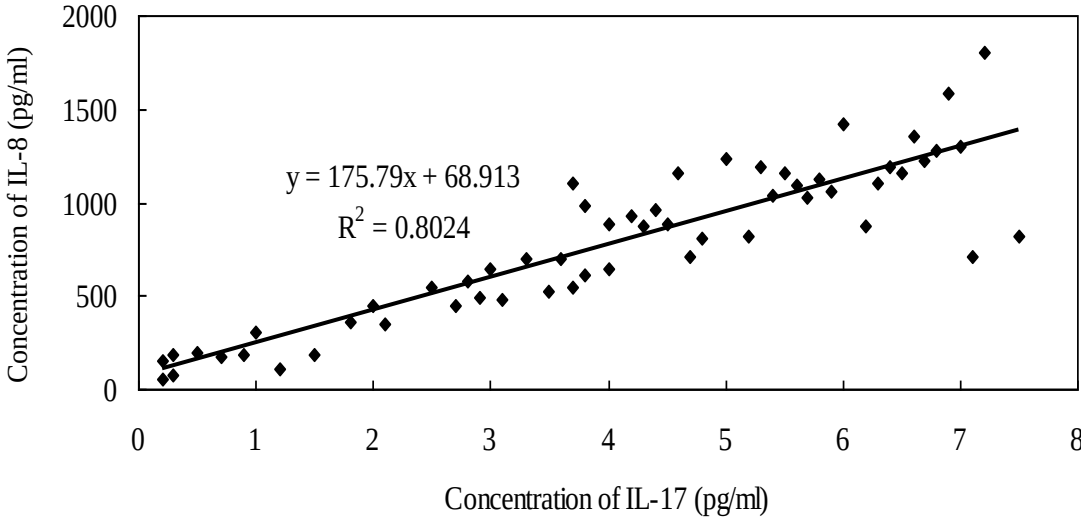

Figure 2. The correlation coefficient of IL-17 and IL-8.

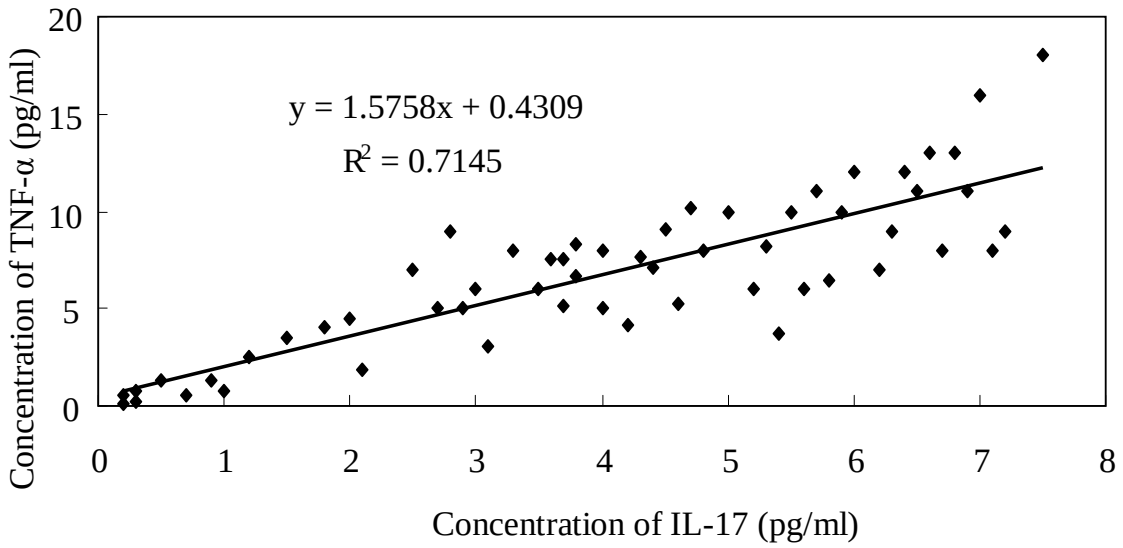

Figure 3. The correlation coefficient of IL-17 and TNF-α.

and infertile) and found that IL-17 level is negatively related to sperm motility and positive to viability, but is not related to density, morphology, and seminal pH. In addition, the sperm mortality rates of high IL-17 samples were significantly higher than those of low IL-17 samples at all time points ($P<0.05$). L-17 induces stromal cells to secrete IL-6, IL-8, and promotes macrophages to release IL-1β, IL-6, IL-10, IL-12 and TNF-α (Takaya et al., 2002), and this report also shows that seminal IL-17 level is positively correlated well to IL-6, IL-8, and TNF-α levels. Hill et al. (1987) reported that IFN and TNF affected sperm motility and forward move rate by culturing sperm in medium with IFN and TNF and Eisermann et al. (1989) also found that recombinant TNF-α reduced sperm motility 4 to 21 h after adding to the medium. Based on these observations, we suggest that the effects of IL-17 on sperm quality are the results of combined actions of a variety cytokines, and the mechanism needs to be further investigated.

Semen fluid bacteria colony count and IL-17 level

IL-17 is a proinflammatory cytokine with strong inflammatory inducing activity, and promotes the development of inflammation, immune response, and other functions. It has been shown that seminal pathogenic bacteria (such as *E. coli*, etc.) are not correlated to interleukin level (Eggert-Kruse et al., 1995). Bacterial infection is not the only cause of leucocyte sperm disease, which could also be induced by viruses. Studies have shown that increasing serum IL-8 level is positively related to CMV infection (Humar et al., 1999). Here we found that IL-17 levels show no significant differences between high colony counts samples and low colony counts samples of bacteria, which suggests seminal bacteria counts are independent of IL-17 levels.

IL-17 level and levels of IL-6, IL-8 and TNF-α in semen fluid

Like IL-6, IL-8, and TNF-α, IL-17 is a proinflammatory cytokine, which induces stromal cells to secrete IL-6, IL-8, and promotes macrophages to release IL-1β, IL-6, IL-10, IL-12, and TNF-α (Dousset et al., 1997). Here we observed that both IL-6 and Il-8 concentrations are significantly lower in low IL-17 samples than those in high IL-17 samples ($P<0.01$), while TNF-α concentration remains the same ($P>0.05$). Correlation analysis indicates that seminal fluid IL-17 and IL-6, IL-8, TNF-α were positively correlated.

Seminal fluid IL-17 level and plasma FSH, LH, T concentrations

Some cytokines affect the hypothalamus - pituitary - gonad axis and testis. Human and rat pituitary cells have IL-1, IL-2, and IL-6 receptor expressed, and *in vitro* IL-6 can stimulate pituitary cells to release FSH, LH, and PRL. In addition, fertile sperms can synthesize and secrete IL-1β and TNF-α and regulate sperm functional autocrine positively (Yan et al., 2000). In this report no effect of seminal IL-17 level on the secretions of human reproductive hormones FSH, LH, and T has been observed.

Researchers have focused on the functions of IL-17 on tumor, blood disease, kidney disease and other diseases. However, studies on IL-17 and male reproduction are still lacking. The mechanisms of IL-17 on male fertility need further investigation.

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