

The Relation Between Follicular Fluid Humanin Concentration and Intra Cytoplasmic Sperm Injection Outcome in Some Iraqi Women Underwent Controlled Ovarian Hyper Stimulation Program

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Abstract

Humanin (HN) is 24-amino acid mitochondria-associated peptide. The role for HN has been reported in many biological processes such as apoptosis, cell survival, substrate metabolism, inflammatory response, and response to stressors such as oxidative stress, ischemia, and starvation. The present study analyzes the relationships between Humanin concentration in the follicular fluid of women undergoing ovarian stimulation for Intra Cytoplasmic Sperm Injection (ICSI) and clinical outcomes after it.

There were 127 women included in this study, they underwent controlled ovarian hyper stimulation, program followed by ova pick up, at that time follicular fluid were obtained and frozen till the time of analysis for Humanin, patient injected oocyte were cultured in CO₂ incubator, then embryo grading, embryo transfer, pregnancy rate and Humanin level were determined.

Follicular fluid Humanin was significantly higher in pregnant females ($p < 0.001$), there was significant negative correlation between mean patients age and follicular fluid Humanin ($r = -0.320$, $p < 0.001$).

Keywords: Humanin, Follicular Fluid, Intra Cytoplasmic Sperm Injection (ICSI).

Introduction

Humanin (HN) is a 24-amino acid mitochondrial peptide, which was exposed in 2001 by Hashimoto due to its protecting influence against amyloid beta toxicity in Alzheimer's disease⁽¹⁾. Humanin is an open reading frame (ORF) originate within the 16s rRNA gene, Like a Russian nesting toy, Humanin is a gene within a gene within a genome of an organelle within a cell^(2,3). The discovery of Humanin is exciting, it is a small, secreted 24 or 21 amino acid peptide, dependent on cytoplasmic or mitochondrial translation correspondingly, with biological action that doesn't have a signal peptide for secretion but may act as a signal peptide itself⁽⁴⁾.

Humanin has been generally identified in different adult tissues with high metabolic rate, including skeletal and cardiac muscle, liver, brain, retinal pigment epithelium, blood vessels, pancreatic beta cells tumors and testes^(5,6,7). (Levels of HN are measurable in plasma, cerebrospinal fluid (CSF), and seminal fluid indicating that it is a secreted protein, though it is still unclear which tissue(s) contributes to the circulating HN pool^(8,9)).

Humanin is present in the human ovary and can be secreted to the follicular fluid⁽¹⁰⁾. Circulating levels of HN decline with age in mice and humans, indicating age dependent regulation of its expression⁽¹¹⁾. Humanin was known for its *anti-apoptotic activity*, which is mediated by HN binding to Bcl-2 associated X protein (Bax), a member of the B-cell lymphoma-2 (Bcl-2) family which regulates cellular survival⁽¹²⁾.

Humanin also interacts with insulin-like growth factor-binding protein 3 (IGFBP3), where it can block IGFBP3-induced apoptosis in glial cell lines⁽¹³⁾. the anti-apoptotic effect of Humanin was specific to BAX-dependent apoptosis, as apoptosis by a BAX-independent stimulus, such as the use of tumor necrosis factor, was not suppressed⁽¹⁴⁾.

Follicular fluid (FF) has recently emerged as an essential target towards investigation of subfertility factors and IVF failure, since it is directly involved in the development and maturation of the oocyte, providing a nourishing intrafollicular microenvironment⁽¹⁵⁾

The present study analyze the relationships between Humanin concentration in the follicular fluid of women undergoing ovarian stimulation for ICSI and clinical outcomes after ICSI.

Methods

A prospective study was conducted in Kamal AL-Samarae hospital and Al Nada private center for Infertility Diagnosis and Assisted Reproductive Technologies, Baghdad/Iraq from December 2019 to April 2021, 127 women were included in this study, they underwent controlled ovarian hyper stimulation program, flexible antagonist followed by ova pick up, at that time follicular fluid were obtained and frozen till the time of analysis for Humanin level, patient injected oocyte were cultured in CO2 incubator, followed by embryo grading, embryo transfer, pregnancy checking by determining serum hCG level. pregnancy rate and Humanin level were determined.

Results

The patients baseline hormones was shown in table 1. Follicular fluid Humanin was significantly higher in pregnant females ($p < 0.001$ as illustrated in table 2), there was significant negative correlation between mean patients age and follicular fluid Humanin ($r = -0.320$, $p < 0.001$, figure 1) and significant negative correlation between follicular fluid Humanin with serum FSH and LH ($r = -0.325$, $p < 0.001$) and ($r = -0.278$, $p = 0.002$) respectively, Humanin was also significantly and positively correlated with serum AMH ($r = 0.357$, $p < 0.001$) table 3.

Discussion

Follicular fluid configuration strongly effects oocyte quality, developmental fitness and the quality of the following embryo^(16,17) (For this aim, many studies have emphasized follicular fluid as an significant cause of potential non-invasive biomarkers for oocyte and embryo quality, as well as for medical result guess.^(18,17)

This study showed that patients with greater Humanin levels expert an increased fortuitous of clinical pregnancy. Humanin shows an important role in preserving the structural and functional

homeostasis of mitochondria, and is elaborate in mitochondria-dependent apoptosis, oxidative stress and energy metabolism^(19,20). As all of these processes have been shown to distress oocyte growth and maturation obviously^(21,22,23) Humanin in the follicles may also act to defend granulosa cells from apoptosis, weighing scale oxidative stress in the microenvironment of follicular fluid, and recover the metabolic eminence of oocytes via tools similar to those in other organs. Consequently, greater levels of Humanin may increase the fertilization rate and encourage succeeding clinical pregnancy

Follicular fluid Humanin levels were correlated with ovarian reserve markers, and were associated with clinical pregnancy for women undergoing IVF–ICSI. It has been demonstrated that Humanin exerted the cytoprotective effect via an intracellular, autocrine, or paracrine mechanism^(4; 24).

Our further analysis showed that Humanin concentration in the follicular fluid was negatively associated with patients age, FSH and LH, and positively associated with AMH, all of which are useful predictors of ovarian reserve^(25,26) indicating an association between Humanin and ovarian function and fertility. No obvious relationship, however, was observed between follicular fluid Humanin levels and BMI, which have also been reported to affect ovarian function⁽²⁷⁾ (May-Panloup et al., 2016). Apart from the association between Humanin and age, Meng et al 2019 reach the same result as this study regarding Humanin and ovarian reserve marker.

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Table 1: patient baseline hormonal levels.

Hormone	GMCSF media group n=30
FSH (mIU/ml)	6.92 ± 1.07
LH (mIU/ml)	4.99 ± 1.21
AMH (ng/ml)	1.60 ± 0.51
E2 (pg/ ml)	42.30 ± 16.93
Prolactin(ng/ml)	7.71 ± 2.93
TSH(mIU/ml)	2.29 ± 0.86

SD: Standard deviation ; FSH: Follicle stimulating hormone ; LH : Luteinizing hormone; E2: Estradiol ; AMH: antimullerian hormone; TSH: Throid stimulating hormone.

Table 2: Humanin level in pregnant and non pregnant patients

Parameters	Pregnant females n=48	Non-pregnant females n=79	p value
F.F Humanin(pg/ml)	148.15 ± 97.59	97.22 ± 62.35	< 0.001*

F.F :follicular fluid; *:p value < 0.05 (significant)

Table 3: Correlations between Humanin levels with hormonal levels

Parameters		Follicular fluid Humanin
AMH	r	0.357
	p value	<0.001*
FSH	r	-0.325
	p value	<0.001*
LH	r	-0.278
	p value	0.002*
TSH	r	-0.132
	p value	0.389
E2	r	-0.059
	p value	0.706
Prolactin	r	-0.049
	p value	0.754

FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; AMH: antimullerian hormone; TSH: Thyroid stimulating hormones; *: p value < 0.05 (significant); r: Pearson's correlation coefficient.

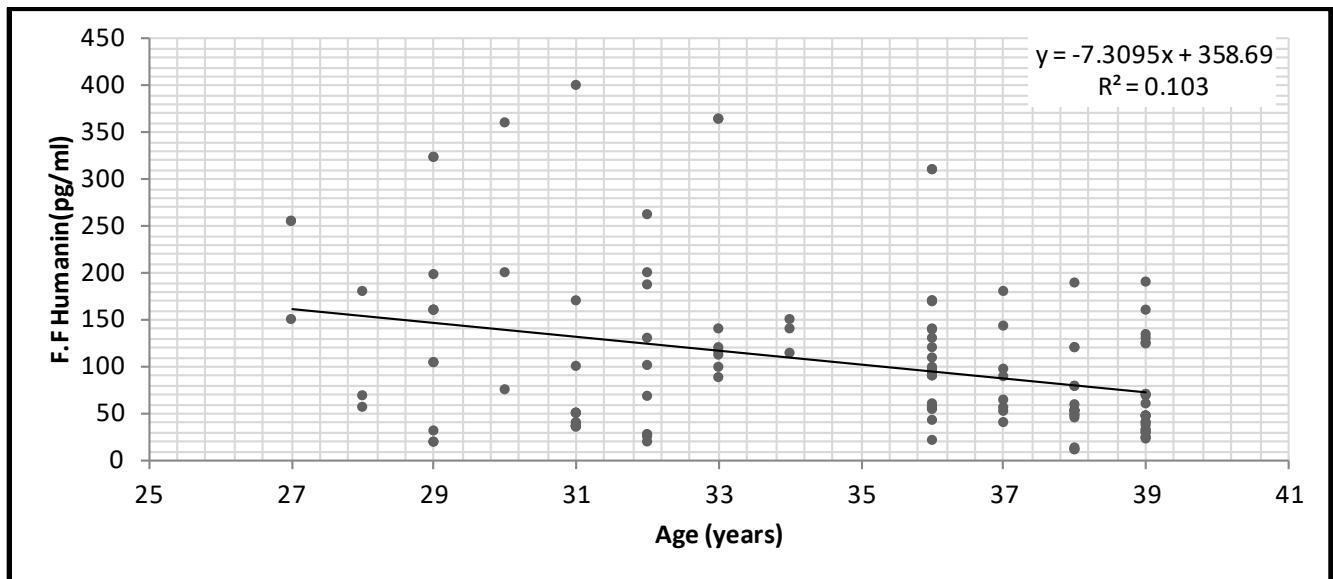


Figure 1: Correlation between patient's age and follicular fluid Humanin