ORIGINAL ARTICLE

Impact of Heat Shock Protein and Tumour Necrosis Factor on Klinefelter Syndrome

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ABSTRACT

Background: Klinefelter Syndrome is the most common sex chromosome aneuploidy (47, XXY), with the existence of an extra chromosome that produces increased levels of gene products and changes in gene expression and contributing to proinflammatory status.

Aim: Identifying the relationship of heat shock proteins and Tumour Necrosis Factor in Klinefelter syndrome pathogenesis.

Methods: This study included 35 Klinefelter syndrome patients, their age ranged from 8-16 years (14.14±1.95). Patients were clinically diagnosed, then karyotype was performed for all patients. Biochemical analyses including heat shock protein 70 (HSP70) and the proinflammatory marker Tumour Necrosis Factor alpha (TNF-α) were performed.

Results: Developmental delay occurred in 48.6% and facial dysmorphism including epicanthal folds, hypertelorism, depressed nasal bridge in 28.6%, gynecomastia in 28.6%, undescended Testis in 60%, increased height in 69.6%, congenital heart disease in 54.3%, intellectual disability in 57.1% and the karyotype was 47, XXY in all patients. The level of HSP70 and TNFα in Klinefelter syndrome patients was higher compared to the normal controls. Moreover, the level of HSP70 and TNF-α in the patients with Klinefelter syndrome and intellectual disability was higher than those without intellectual disability. On the other hand, testosterone level was decreased in KF patients compared to controls. Moreover, a significant negative correlation was observed between testosterone and both HSP70 and TNF-α.

Conclusion: The particular impacts of HSP70 and TNF-α remain to be elucidated in future studies to enlighten their importance and possible association with the severity of Klinefelter syndrome.

Keywords: Chromosomal aberration, Klinefelter's syndrome, heat shock protein 70, Tumor Necrosis Factor, Testosterone.

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INTRODUCTION

Klinefelter's syndrome is a sex chromosomal aberration of male infertility. It is the most common form of aneuploidy with a prevalence estimated to about 1 in 650 newborn boys (1). Patients with Klinefelter syndrome are phenotypically males but carry an extra X chromosome (47,XXY). Interestingly, the existence of an extra chromosome can produce increased levels of gene products and changes in gene expression (2). These studies showed that gain of chromosomes triggers replication stress promoting genomic instability and possibly contributing to proinflammatory status. Patients present with tall stature, cryptorchidism, gynecomastia, cognitive impairment, infertility and hypergonadotropic hypogonadism (3). Klinefelter syndrome is considered to induce a state of chronic proinflammation which may be involved in its pathogenesis (4).

The heat shock protein family (HSPs) play a critical role in avoiding protein misfolding, constraining apoptosis and represent a class of proteins potentially involved in Klinefelter syndrome pathogenesis. HSP70 is expressed in spermatocytes and spermatids in normal and maturation arrest testicular tissues in human (4). However, the expression of HSP70 was shown to be low in maturation arrest, and no HSP70 was demonstrated in Sertoli-only specimens (5). Therefore, the decreased expression of the heat shock protein HSP70 may be associated with the pathogenesis of male infertility and chromosomal aneuploidies (6).

X chromosome contains genes related to immune regulation, suggesting that the number of female chromosomes could play a role in inflammation, influencing the production of inflammatory cytokines. An increased production of inflammatory cytokines expressed by elevated Tumour necrosis factor has been demonstrated in patients with Klinefelter syndrome as compared to eugonadal males (7). The aneuploid state could provoke a proinflammatory condition (8,9).

The present research will focus on HSP70 and Tumour Necrosis Factor alpha (TNF-α) with the aim of identifying their potential relatioinship in the pathogenesis of Klinefelter Syndrome.

MATERIALS AND METHODS

In this cohort were 35 Klinefelter syndrome males, their ages ranging from 8-16 years (14.14±1.95). Patients were referred to the Clinical Genetics Clinics, National Research Centre (NRC), Cairo, Egypt, for diagnosis and counselling. Patients were first diagnosed clinically, then karyotype was performed for all patients. Biochemical analyses including HSP70 and the proinflammatory marker TNF-α were performed.

Samples were collected after obtaining informed written consents from the patients or their parents' according to the Helsinki Declaration of 1975, as revised in 1983, and the Institutional Review Board of the National Research Centre.

Thirty-two healthy children of matching age and sex served as controls. Complete medical history taking, three-generation pedigree construction, clinical examination, anthropometric measurements, echocardiogram, and intelligence quotient (IQ) evaluation using Wechsler intelligence scales were performed (10). In addition, karyotypes were assessed in all patients.

Cytogenetic studies

By the conventional G-banding technique (11,12). At least 25 metaphases were karyotyped and nomenclature according to that cited in reference (13).

Biochemical Assessment

Quantification of Tumour Necrosis Factor –α (TNF-α) was determined using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit purchased from NOVA (Bioneovan, Beijing, China) according to the manufacturer's instructions. The concentration of TNF-α in a sample was determined by interpolation from the standard curve. Quantification of heat shock protein-70 (HSP70) was determined using an ELISA kit from NOVA (Bioneovan, Beijing, China) according to the manufacturer's instructions. The concentration of HSP70 in a sample was determined from the standard curve. Quantification of Testosterone was using an ELISA kit from NOVA (Bioneovan, Beijing, China) according to the manufacturer's instructions.

RESULTS

The clinical findings of our patients are shown in Table 1. The studied patients were from different governorates in Egypt. Parental consanguinity was positive in 57.1% of the studied families. The patients experienced developmental delay in 48.6% and facial dysmorphism including epicanthal folds, hypertelorism, depressed nasal bridge in 28.6%, gynecomastia

in 28.6%, undescended Testis in 60%, increased height in 69.6%, congenital heart disease in 54.3%, intellectual disability in 57.1% and the karyotype was 47, XXY in all patients (Figure 1).

Table 1. The clinical data of the Klinefelter syndrome patients

	Number N=35	Percentage (%)
Age in years	8-16	(Mean±SD:14.14±1.95)
Consanguinity	20/35	57.1
Developmental Delay	17/35	48.6
Dysmorphism	10/35	28.6
Gynecomastia	10/35	28.6
Undescended Testis	3/35	60
Increased Height	24/35	69.6
Congenital Heart Disease	19/35	54.3
Intellectual Disability	20/35	57.1
Karyotype	47, XXY	100

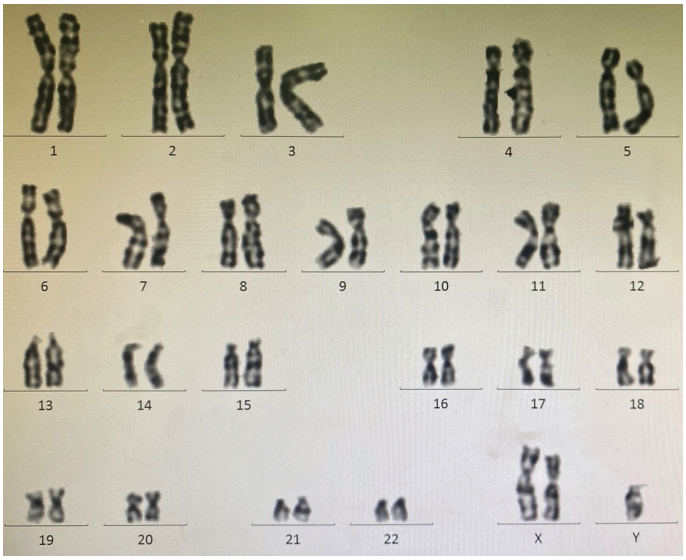

Figure 1. A karyogram from a Klinefelter syndrome patient (47, XXY).

Table 2. HSP70, TNF-α and testosterone in the Klinefelter syndrome patients and controls

Parameters	KF	Control	P
HSP70 (ng/mL)	91.33±14.514	60.84±18.67	< 0.001
TNF-α (pg/mL)	150.53±15.6078	101.11±15.39	< 0.001
Testosterone (ng/mL)	3.04± 0.89	7.00 ±1.15	< 0.001

The HSP70 and TNF-α in the Klinefelter syndrome patients was significantly higher compared to the normal controls.
While the testosterone level was significantly lower in patients than controls.

Table 3. Correlation between serum testosterone levels and HSP70 and TNF-α in Klinefelter patients.

		Heat Shock Protein 70	TNF-α
Testosterone	r	-0.847	-0.880
	p	0.001	0.001

The high level of HSP70 and TNF-α in the patients with Klinefelter syndrome correlated with the low level of testosterone.

Table 4. Mean of HSP70 and TNF-α in Klinefelter patients with no intellectual disability and with intellectual disability.

Parameters	No Intellectual Disability	Intellectual Disability	p
HSP70 (ng/mL)	78.67±4.163	104.00±5.29	0.003
TNF-α (pg/mL)	139.71±3.51	161.35±15.67	0.05

The level of HSP70 and TNF-α in the patients with Klinefelter syndrome and intellectual disability was higher than those without intellectual disability.

DISCUSSION

Klinefelter's syndrome is the most common chromosomal aneuploidy (14), and is usually underdiagnosed, with only 25% of the patients being diagnosed prior to puberty. Most Klinefelter syndrome patients are diagnosed in adulthood owing to the effects of hyper- gonadotropic hypogonadism (15). The clinical data of the studied Klinefelter syndrome patients is summarized in Table 1. In the current study we diagnosed these patients in childhood (8-16 years). 57.1% of the patients were the offspring of consanguineous marriage, Vallabhajosyula et al., (16) reported consanguinity in 22.2% of their patients. Developmental delay occurred in 48.6% of the patients although Tartaglia et al., (3) reported developmental delay in 35% of his Klinefelter syndrome patients. This may be owing to his larger cohort and differences in age. Facial dysmorphism including epicanthal folds, hypertelorism, depressed nasal bridge was identified in 28.6%, our study is in agreement with previous investigators (17,18). Furthermore, it is worth noticing that gynecomastia, undescended testis and tall stature are hallmarks of Klinefelter syndrome, and their recognition could improve the rate of early diagnosis. Gynecomastia was present in 28.6% of our patients. Similarly, Chang et al., (19) identified that gynecomastia was found in 28% of their patients. In the current study, undescended testis was detected in 60% of the studied patients. Bojesen et al., (20) reported cryptorchidism in 37% of Klinefelter syndrome patients (21). Moreover, tall stature was observed in 69.6% of the studied patients. Our findings are in agreement with former studies elucidating that the presence of three copies of the SHOX gene on the X-chromosome, a possible effect of X chromosome gene dosage explains excess height in KS (15,19). Moreover, congenital heart disease (CHD) was revealed in 54.3%. Claus et al., (15) noted CHD in the form of mitral valve prolapse in 50%. Additionally, intellectual disability was assessed in 57.1% of the patients. Similarly, Simonetti et al., (21) recorded intellectual disability present in Klinefelter syndrome which relied on an imbalance in X-chromosome gene expression. On the other hand, it was reported that the majority of subjects with 47, XXY karyotype have a normal intellectual level (22).

In our study, the karyotype (47, XXY) confirmed the clinical diagnosis of Klinefelter syndrome in all patients (Figure1.). This is consistent with previous investigators (15,17,21). The biochemical findings of the patients are summarized in Table 2 and 3.

Our findings revealed a statistically significant variance between cases and controls in terms of HSP70. Previous studies concluded that high level of HSPs may enhance the pathogenesis

of male infertility (6). Increased serum HSP70 suggests oxidative stress, systemic inflammation, and maintenance of immune homeostasis which is likely due to the supernumerary X chromosome that contributes to several pathologies in Klinefelter syndrome (23,24). Furthermore, we recorded higher levels of the inflammatory cytokines TNF-α in Klinefelter syndrome patients, in relation to the controls. This is consistent with Lefevre et al., (25) who claimed that the excess number of X chromosomes influences inflammatory cytokine production. This emphasises the view that chronic low-grade inflammation is involved in the pathogenesis of Klinefelter syndrome. The high level HSP70 in the patients with Klinefelter syndrome correlated with the low level of testosterone in our patients. Recent studies indicated that HSP70 have a role in testicular damage (26). Thus, testosterone treatment has been the keystone in the care for patients with Klinefelter syndrome.

Moreover, the high level of TNF-α in the patients with Klinefelter syndrome correlated with the low level of testosterone, this is consistent with the previous research that stated that testosterone attenuates the peripheral inflammatory process by inhibiting the expression and function of the inflammatory cytokines TNF-α (27).

Interestingly, our results demonstrated significant differences in HSP70 in Klinefelter syndrome with intellectual disability and those with normal mentality (Table 4). Additional evidence has indicated that accumulation of damaged protein aggregates and dysfunction of intracellular degradative system are orchestrating the neurodegenerative processes (28,29). We suggest that the accumulation of the HSP70 in Klinefelter syndrome which plays a role in neuronal development could contribute to the intellectual disability.

The inflammatory cytokine TNF-α was observed significantly elevated in the patients with Klinefelter syndrome with intellectual disability than those without intellectual disability (Table 4). Previous research (30) has demonstrated that TNFα is associated in the pathogenesis of neurodegenerative disorders and cognitive dysfunction. Cytokine dysregulation may highlight the clinical outcome and raised pro-inflammatory biomarkers appear to be an important feature in Klinefelter syndrome. This is supported, in part, by dos Santos et al, who have identified the interactions of HSP70, TLR4 and TNF-α in pathological processes including binding to dendritic cells and mediating cellular response (31).

In conclusion, the particular impacts of these biomarkers remain to be elucidated in future studies to enlighten their importance and possible association with the severity of Klinefelter syndrome.

AUTHOR CONTRIBUTIONS

To the conception and design of the study, all authors made contributions. The biochemical, cytogenetic, and statistical studies were carried out by (Moushira Zaki), (Eman R. Youness), (Heba A. Elmalt), and (Azzah A. Khedr). Fatma Abdelrahman Alzaree and Mohammed M. EL-Sonbaty prepared the material and collected the data. Hala T. El-Bassyouni wrote the manuscript's initial draft; further drafts were authored by all other contributors. The final manuscript was read and approved by all writers. This article was published with the study's generated or analyzed data.

DECLARATIONS OF INTEREST

The authors confirm that this manuscript has been submitted as a “Pre-print” to “Research Square” and is on-line at https://doi.org/10.21203/rs-2496942/v1 and is classed by that site as a preliminary report that has not undergone peer review.

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REFERENCES

1. Los E, Ford GA. Klinefelter Syndrome. (Updated 2022 May 1). In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2022 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482314.
2. Belling K, Russo F, Jensen AB, et al. Klinefelter syndrome comorbidities linked to increased X chromosome gene dosage and altered protein interactome activity. *Hum Mol Genet* 2017; 26(7): 1219-1229.
3. Tartaglia N, Cordeiro L, Howell S, et al. The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter syndrome). *Pediatr Endocrinol Rev* 2010; 8 Suppl 1(0 1):151-159.
4. Fukuhara S, Mori J, Nakajima H. Klinefelter syndrome in an adolescent with severe obesity, insulin resistance, and hyperlipidemia, successfully treated with testosterone replacement therapy. *Clin Pediatr Endocrinol* 2021; 30(3): 127-132.
5. Sabeti P, Amidi F, Kalantar SM, et al. Evaluation of intracellular anion superoxide level, heat shock protein A2 and protamine positive spermatozoa percentages in teratoasthenozoospermia. *Int J Reprod Biomed* 2017; 15(5): 279-286.
6. Feng HL. Molecular biology of male infertility. *Arch Androl* 2003; 49: 19-27.
7. Aliberti L, Gagliardi I, Lupo S, et al. Investigation of COVID-19 infection in subjects with Klinefelter syndrome. *J Endocrinol Invest* 2022; 45(5): 1065-1069.
8. Yoshiuchi I, Itoh N, Nakano M, et al. Case report of Klinefelter's syndrome with severe diabetes, dyslipidemia, and stroke: The effect of pioglitazone and other anti-inflammatory agents on interleukin-6 and -8, Tumour necrosis factor-alpha, and C-reactive protein. *Diabetes Care* 2006; 29(8): 1981.
9. Zhu J, Tsai HJ, Gordon MR, Li R. Cellular Stress Associated with Aneuploidy. *Dev Cell* 2018; 44: 420-431.
10. Watkins MW, Beaujean AA . Bifactor structure of the Wechsler Preschool and Primary Scale of Intelligence–Fourth Edition. *Sch Psychol Q* 2014; 29: 52–63.
11. Seabright MA. Rapid banding technique for human chromosomes. *Lancet* 1971; 2(7731): 971-972.
12. Verma RS, Babu A. Human chromosomes principles and techniques 2nd Edition, NewYork, McGraw-Hill, 1995.
13. An international system for human cytogenetic nomenclature. *ISCN* 2016; Editors: McGowan-Jordan J, Simomns A, Schmid M. Basel: Karger Publishers.
14. Madian A, Eid MM, Shahin AAB, et al. Detection of low-

grade mosaicism and its correlation with hormonal profile, testicular volume, and semen quality in a cohort of Egyptian Klinefelter and Klinefelter-like patients. *Reprod Biol* 2020; 20 (2): 259-263.
15. Claus H Gravholt, Simon Chang, Mikkel Wallentin, et al. Klinefelter Syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev* 2018; 39: 389–423.
16. Vallabhajosyula R, Rajangam S, Lalitha C. Association of parental origin with clinical profile in Klinefelter syndrome. *J Clin Diagn Res* 2015; 9: GC01-3.
17. Mazen I, El-Ruby M, El-Bassyouni HT. Variable associations of Klinefelter syndrome in children. *J Pediatr Endocrinol Metab* 2010; 23(10): 985-989.
18. Purnak S, Ada S, Güleç AT, et al. Diagnosis of variant Klinefelter syndrome in a 21-year-old male who presented with sparse facial hair. *Ann Dermatol* 2012; 24 3): 368-369.
19. Chang S, Skakkebæk A, Trolle C, et al. Anthropometry in Klinefelter syndrome--multifactorial influences due to CAG length, testosterone treatment and possibly intrauterine hypogonadism. *J Clin Endocrinol Metab* 2015; 100(3): e508-517.
20. Bojesen A, Juul S, Birkebaek NH, et al. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. *J Clin Endocrinol Metab* 2006; 91(4):1254-1260.
21. Simonetti L, Ferreira LGA, Vidi AC, et al. Intelligence Quotient Variability in Klinefelter Syndrome Is Associated with GTPBP6 Expression Under Regulation of X-Chromosome Inactivation Pattern. *Front Genet* 2021; 12: 724625.
22. Verri A, Cremante A, Clerici F, et al. Klinefelter's syndrome and psychoneurologic function. *Mol Hum Reprod* 2010; 16(6): 425-33.
23. Qu B, Jia Y, Liu Y, et al. The detection and role of heat shock protein 70 in various non disease conditions and disease conditions: a literature review. *Cell Stress Chaperones* 2015; 20(6):885-892.
24. Jannatifar R, Cheraghi E, Nasr-Esfahani MH, et al. Association of heat shock protein A2 expression and sperm quality after N-acetyl-cysteine supplementation in asthenoterato-zoospermic infertile men. *Andrologia* 2021; 53(5): e14024.
25. Lefèvre N, Corazza F, Valsamis J, et al. The number of X chromosomes influences inflammatory cytokine production following toll-like receptor stimulation. *Front Immunol* 2019; 10: 1052.
26. Arun S, Chaiyamoon A, Lapyuneyong N, et al. Chronic stress affects tyrosine phosphorylated protein expression and secretion of male rat epididymis. *Andrologia* 2021; 53: e13981.
27. Yang L, Zhou R, Tong Y, et al. Neuroprotection by dihydrotestosterone in LPS-induced neuroinflammation. *Neurobiol Dis* 2020; 140: e104814.
28. Di Domenico F, Coccia R, Cocciolo A, et al. Impairment of proteostasis network in Down syndrome prior to the development of Alzheimer's disease neuropathology: redox proteomics analysis of human brain. *Biochim Biophys* Acta 2013; 1832: 1249-59.
29. Patra M, Weiss C, Abu-Libdeh B, et al. A novel variant of the human mitochondrial DnaJ protein, Tid1, associates with a human disease exhibiting developmental delay and polyneuropathy. *Eur J Hum Genet* 2019; 27: 1072-1080.
30. Zhuang Y, Xu HC, Shinde PV, et al. (2020) Fragile X mental retardation protein protects against tumor necrosis factor-mediated cell death and liver injury. Gut 2020; 69: 133-145.
31. Dos Santos RS, Veras FP, Ferreira DW. et al Involvement of the hsp70/TLR4/IL6 and TNF-α pathways in delayed onset musclae soreness. *J Neurochem* 2020; 155: 29-44.

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