



Successful Treatment of Sperm DNA Fragmentation Through Ayurveda Rasayana Therapy: A Case Study

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Abstract

Background: Sperm DNA fragmentation (SDF) can affect fertilization rate and embryo development, making it a useful measure for assessing male fertility. Available evidence supports the association between high sperm DNA fragmentation and poor outcomes, with regard to natural conception. Several treatment options are being adopted with varying degrees of success. Some of the commonly used treatment options are the intake of oral antioxidants, varicocele repair, and techniques like micro-manipulation-based sperm selection and use of testicular sperm for intracytoplasmic sperm injection.

Case Presentation: Studies have shown that around 29% of couples depend on complementary and alternative medicine (CAM) modality for the treatment of infertility. However, there is a lack of substantial evidence regarding its efficacy in treating various aspects of infertility in couples. The current case report is about a 44 year-old male patient with infertility, who has a known diagnosis of sex chromosome abnormalities. Meanwhile, the SDF study reports indicated the presence of chromosomal abnormalities. This patient was treated exclusively with Ayurveda therapy aimed towards qualitative improvement in reproductive tissues (Shukra Dhatu as per Ayurveda). Patient was assessed periodically for changes in chromosomal abnormality. After four months of treatment, the evaluations demonstrated the presence of completely normal chromosomes.

Conclusion: This case study indicates the potential of Ayurveda therapy in treating cases of male infertility caused by DNA fragmentation. Furthermore, observations and systematically designed clinical trials are warranted to establish a stronger level of evidence before making further clinical recommendations.

Keywords: Complementary therapies, DNA fragmentation, Integrative medicine, Male infertility.

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Introduction

Infertility is one of the leading health concerns worldwide and this term is defined as the inability to achieve pregnancy within 12 consecutive months of unprotected intercourse (1). According to global surveys, approximately 20% of infertility cases are associated with male factors (2). The integrity of both parental genomes

is very crucial for normal fertilization, implantation, maintenance of pregnancy, and fetal development. Sperm DNA fragmentation (SDF) causes loss of sperm DNA integrity leading to infertility or improper fetal development (3). Both testicular and post-testicular mechanisms are implicated in the etiology of SDF affecting natural as well as

assisted reproduction. An increasing number of reports suggest a direct relationship between SDF and male infertility (4). Several factors including varicocele, oxidative stress, genetic abnormalities, systemic diseases, altered lifestyle, and exposure to xenobiotics can influence SDF (5, 6). Poor-quality sperm chromatin structure increases the risk of early pregnancy loss after in vitro fertilization and intracytoplasmic sperm injection (2).

Nowadays, lifestyle modifications, infection control, oral antioxidant therapy, varicocele repair, and micromanipulation-based sperm selection are majorly used to treat SDF (2). Oxidative stress is known to damage sperm DNA. Thus, oral antioxidants are used to reduce oxidative stress, thereby repairing DNA damage; however, the effect is arguable as DNA damage persists in patients even after treatment (7). It was reported that higher levels of SDF are observed in patients with varicocele. Varicocele repair often involves microsurgical varicocelectomy (8). Micromanipulation-based sperm selection methods are also used although there is no definite clinical evidence that these methods can prevent the harmful effects of abnormal sperm. The limitations associated with these methods may result in a lack of success in mainstream fertility treatments (9). The use of complementary and alternative medicine (CAM) is thus increasing in infertility treatment. Studies have shown that around 29% of couples utilize a CAM modality for the treatment of infertility (10). However, there is a paucity of evidence related to its efficacy in treating various aspects of infertility in couples.

In this case study, a 44 year-old male patient was reported who sought treatment for infertility associated with sex chromosome abnormalities. The patient received exclusive Ayurveda therapy as part of his treatment. He showed complete normal chromosomes in a span of around four months of treatment. In this case, the Ayurvedic perspective suggests the involvement of vitiated Shukra Dhatu (reproductive tissue). Therefore, the chosen treatment approach for this patient focused on enhancing the quality of Shukra Dhatu as herbs were reported to improve sexual vigor and promote sperm quality and quantity (11). Hence, such type of herbs and medicinal formulations with Vajikaran (Aphrodisiac) and Rasayana (Adaptogenic) properties were selected.

According to Ayurveda principles, this case was classified as a deformity in Shukra Dhatu (reproductive tissues) and was treated accordingly. The

therapeutic approach focused on correcting this deformity through treatments aimed at enhancing the quality of Shukra Dhatu. In this patient, specific formulations with pharmaceutical activities were selected. The formulations include herbo-mineral Ayurvedic preparations such as Heerak Bhasma (incinerated ash of biologically processed diamond), Bramha Rasayana (a paste formulation of adaptogenic herbs), Vrushya Ghrita (aphrodisiac herbs processed with milk fats), and Sukumar Ghrita (adaptogenic herbs processed with milk fats). Heerak Bhasma is known to have nanoparticles and has shown its activity in the DNA damage (12). It is also used in the targeted drug delivery and sustained drug release to boost therapeutic efficacy and improve safety profile (13). Bramha Rasayana has shown an inhibitory effect on mutagen induced chromosomal anomalies (14). The outcomes of this study suggest the potential efficacy of Ayurveda therapy treatment of male infertility associated with DNA fragmentation.

Case Presentation

A 44 year-old male patient diagnosed with hypospermia and teratozoospermia visited the clinic in August 2020 for treatment of infertility. The patient had a medical history of hypertension, spanning 5-6 years, and he had been taking prescribed medication for its management. Additionally, the patient had been taking Levothyroxine (Thyronorm) for the past 2 years to manage hypothyroidism. During the course of treatment, both hypertension and hypothyroidism were affectively managed. The patient reported experiencing a stressful work environment and an inactive lifestyle. Before the initiation of treatment, the patient was a non-smoker and did not consume alcohol. Throughout the course of treatment, there were no reported changes in his sedentary lifestyle. His main complaints were erectile dysfunction for 3-4 months and loss of libido. In addition to the aforementioned symptoms, the patient also experienced frequent sleep disturbances and mood swings. Baseline laboratory tests were carried out, revealing the following values: thyroid stimulating hormone (TSH) -11.23 *mIU/ml* (reference range: 0.27–4.20 *mIU/ml*), vitamin B12 -182.90 *pg/ml* (reference range: 200-900 *pg/ml*), free testosterone -4.98 *ng/dl* (reference range: 5.88–18.35 *ng/dl*), sex hormone binding globulin (SHBG) -24.18 *nmol/L* (reference range: 14.5 to 48.4 *nmol/L*), and total testosterone -226.90 *ng/dl* (reference range: 249-836 *ng/dl*). Considering the patient's laboratory

reports and history, Ayurveda Rasayana therapy along with Panchakarma therapy was administered for improvement in seminal parameters. The patient's previous medications remain unchanged during the entire therapy. After eight months of the above treatment, there was an improvement in seminal parameters like enhanced liquefaction, increased sperm concentration, and improved total motility. Despite the observed improvement in seminal parameters, the patient's partner was unable to conceive. As a result, the patient was advised to undergo a DNA fragmentation study. It was carried out by using fluorescence in-situ hybridization (FISH) method. FISH was performed on sperm nuclei using FISH aneuploidy detection probes targeting chromosomes 13q14.2 (green), CEP 18 (aqua), 21q22.13 region (red) as well as the sex chromosomes including chromosome X (centromere Xp11.1-q11.1) and chromosome Y (centromere Yp11.1-q11.1) (MetaSystems, Germany). The initial DNA fragmentation study reports, dated 05-04-2021, revealed abnormalities in the chromosomes. The same test was prescribed again after the completion of the treatment, and the subsequent DNA fragmentation study was conducted on 29-07-2021. The patient's partner was 40 years old and had undergone evaluation, revealing a history of polycystic ovarian disease (PCOD).

Treatment protocol: Based on the reports of the DNA fragmentation study, the treatment was then planned again, concentrating on the correction of DNA fragmentation. The basic line of treatment was planned as per the Rasayana principle of Ayurveda. Taking into consideration the potential benefits of Rasayana therapy, a study was planned utilizing oral formulations of the treatment. The oral Rasayana formulations with anti-aging properties and those known to target Shukra Dhātu (the reproductive tissues) were carefully selected for the study (Table 1). The Panchakarma therapy for the study included Yoga Basti and Matra Basti, which are both forms of medicated retention enema (Table 2).

After approximately four months of treatment, the DNA fragmentation assessment reports indicated normal results, which are summarized in table 3. To monitor the safety of this therapy, the patient was evaluated for hematological and biochemical tests. Findings after four months of therapy such as renal and kidney function test (RFT and LFT) results are summarized in table 4.

Discussion

Although there are many factors contributing to male infertility such as genetic abnormality, lifestyle choices, and variety of medical comorbid conditions, among others, it is often observed that

Table 1. Details of Rasayana therapy used in treatment of male infertility

Therapy	Details	Time of administration	Administered with
Heeraka Rasayana	Ayurveda formulation primarily containing Heeraka Bhasma <i>i.e.</i> , incinerated diamond ash	Twice a day	Honey
Arpisa Rasayana	Proprietary Ayurvedic formulation (Appendix 1)	Empty stomach at morning	Honey
Brahma Rasayana	Classical Ayurveda formulation (Appendix 2)	Twice a day	Honey
Powder of classical Ayurveda Medicine	Chandraprabha 250 mg, Shankhavati 250 mg, Gokshur (Tribulus Terrestris) 250 mg, Kaunch Beej (Mucuna Pruriens) 250 mg, Rasapachak (A classical herbal formulation containing equal quantity of Indrayav (Holarrhena Pubescens), Patol (Trichosanthes Dioica), Kutki (Picrorhiza Kurroa) 250 mg, Ashwagandha (Withania Somnifera) 250 mg, Bala (Sida cordifolia) 250 mg, and Abhrak Bhasma (Biotite Mica) 125 mg	After meals thrice a day	Warm water
Vrishya Ghrita	Classical Ayurveda formulation (Appendix 3)	Twice a day 10 ml each time	Warm water
Sukumara Ghrita	Classical Ayurveda formulation (Appendix 4)	Twice a day 10 ml each time	Warm water

Table 2. Details of the Panchakarma schedule and dosages used in treatment of male infertility

Panchakarma	Duration	Days	Treatment content
Matra Basti	1 month	Alternate day	Dasha Mash 60 ml
	October 2020		
Yoga Basti	March 2021	8 days	Anuvasan-Dashamoola and Bala Taila 120 ml (1:1)
	June 2021		Nirooh-Erandmuladi Yapan Basti
	July 2021		
Nasya	May 2021	1 month	Ksheerabala Taila

Table 3. Summary of sperm DNA fragmentation test before and after Rasayana treatment

Type of sperm aneuploidies	Case values (%)	Results	Case values (%)	Results	Reference range
	05/04/2021		29/07/2021		
% Disomy of chromosome 13	0.10	Normal	0.05	Normal	0.01%–0.40% *
% Disomy of chromosome 18	0.10	Normal	0.00	Normal	
% Disomy of chromosome 21	0.15	Normal	0.15	Normal	
% Sex-chromosome disomy	1.90	Abnormal	0.30	Normal	
% Diploidy	0.10	Normal	0.00	Normal	

* Biological reference intervals

Table 4. Safety parameters

Parameters	Value	Unit
Liver function test (LFT)		
Total bilirubin	0.39	mg/dL
Bilirubin-conjugated	0.15	mg/dL
Bilirubin-unconjugated	0.24	mg/dL
SGPT	31.4	U/Lt
SGOT	22.5	U/Lt
ALP	88	U/Lt
GGTP	47	U/Lt
Total protein	7.0	g/dL
Albumin	4.7	g/dL
Globulin	2.3	g/dL
Albumin/globulin ratio	2.04	Ratio
Renal function test (RFT)		
Urea	17.70	mg/dL
Sodium	141.0	mmol/Lt
Potassium	4.10	mmol/Lt
Chloride	103.90	mmol/Lt
Creatine	0.8	mg/dL

ALP: Alkaline phosphatase; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase, BUN: Blood urea nitrogen; PT-INR: Prothrombin time-international normalized ratio; GGTP: Gamma glutamyl-transferase

ing the potential contributing factors. The examination of the molecular and genetic factors provides a new direction toward solving infertility problems in males (15). Abnormalities in the sperm chromosomes may result in idiopathic infertility.

The therapy for this patient was planned for qualitative improvement in Shukra Dhatu. The planning of therapy as per Ayurveda began with assessment of the Dosha (the pathological entities) and Dushya (the affected tissue). After evaluation of these pathophysiological factors involved in the patient, treatment was planned targeting the vitiation or deformities in these factors. In this particular case, all three Doshas (Vata, Pitta, Kapha) were involved, and the affected tissue (Dushya) was Shukra Dhatu. Based on this pathophysiology, Rasayana therapy was considered. Rasayana is a branch of Ayurveda that deals with therapies to address pathology related to aging. As per this principle, the therapy consisted of oral Rasayana medications and Panchakarma therapies (cleansing and detoxifying procedures). Aging is known to cause increased oxidative stress and sperm DNA fragmentation worsens with age. This deterioration is thought to be associated with mitochondrial damage. The high levels of reactive oxygen species (ROS) affect the sperm DNA

infertility persists in certain cases even after treat-

structure and causes its fragmentation. It is associated with the disintegration and destruction of DNA structure, which hinders its ability to fertilize an oocyte, ultimately leading to infertility (16). The present case study demonstrated a reduction in DNA fragmentation levels after undergoing Rasayana treatment for a duration of four months. This indicates the potential of Rasayana therapy which may help in reversing chromosomal defects.

Numerous Ayurvedic plants with Rasayana properties are recognized for their beneficial effects in addressing causes related to infertility. Ayurvedic formulations, including those utilized in Rasayana therapy, incorporate metals in a purified, biocompatible, and calcined form known as Bhasmas (17). In the present case, the herbo-metallic formulations contained detoxified gold and mercury in a purified calcined form. The procedure of preparing Bhasmas is outlined in Ayurveda classical texts where each metal undergoes a purification process known as 'Shodhan' and subsequent incineration referred to as 'Maran'. These are important pharmaceutical processes that make the metals/minerals nontoxic, biocompatible, and transform them into a fine ash known as Bhasma (18). Hematological and biochemical assessments conducted after four months of therapy revealed normal values, indicating that the therapy was safe and well-tolerated by the patient.

Bhasma is an integral part of Ayurveda treatment and has a long history of use. Several studies have demonstrated the safety of Swarna Bhasma and other Ayurveda metals in various preclinical (19, 20) and clinical settings (21). Also, there are numerous published case studies and clinical studies regarding the use of Rasayana therapy and its safety (22, 23). Due to their inclusion in traditional medicine with a long history of use, these Bhasmas are approved as 'Ayurveda medicine' by regulatory authorities in India. As a result, these compounds often deviate from the conventional drug development path and may not undergo the typical phased clinical trials for evaluation. The Yog Basti treatment includes Yapana Basti, which is specifically recommended for promoting longevity, addressing age-related diseases, and managing conditions associated with imbalances in Shukra Dhatu (23).

Observations have indicated that *Mucuna Pruriens* has the ability to rejuvenate the antioxidant defense system in infertile men. Furthermore, it

has been found to enhance semen quality and assist in stress management (24). An *in vitro* study on aqueous leaf extract of *Moringa Oleifera* showed reduced intracellular ROS production, DNA fragmentation, and acrosome reaction in human spermatozoa (25). All of these findings correlate with the treatment employed in the present case, suggesting that Rasayana treatment can be used in the management of male infertility associated with parental DNA damage. According to our records, there were no successful pregnancies reported even after addressing the defects in DNA fragmentation. This could potentially be attributed to presence of other comorbidities in the couple.

Conclusion

The present case study highlights the potential benefit of Ayurvedic Rasayana therapy in the management of male infertility associated with sperm DNA fragmentation which causes DNA damage. This case study provides evidence that the approach may serve as a safe and effective alternative treatment for male infertility cases associated with DNA damage or sperm DNA fragmentation. However, to make further clinical recommendations, it is crucial to obtain a stronger level of evidence through controlled clinical trials.

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Conflict of Interest

Authors of this article declare that they have no conflict of interest.

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