

Long-term follow-up of autologous blood cell transplantation along with platelet rich plasma in treatment of women with vesicoureteral reflux: A clinical trial study

Malihe Keshvari Shirvan ¹, Mahmoud Tavakkoli ², Mahdi Mottaghi ³, Daryoush Hamidi Alamdari ⁴, Atena Aghae ⁵, Seyed Hamed Ghadamgahi Moghadam ², Salman Soltani ^{2*}

¹ Department of Urology, Imam Reza Academic Hospital, Mashhad University of Medical Sciences, Mashhad, IR Iran. ² Kidney Transplantation Complications Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. ³ Students Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. ⁴ Stem Cell and Regenerative Medicine Research Group, Department of Biochemistry, Stem Cell Laboratory, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. ⁵ Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Introduction: Vesicoureteral reflux is a common urological issue. The interventions for low-grade reflux have been evolved from ureteral reimplantation to endoscopic injection of a bulking agent. This study aimed to evaluate the therapeutic efficacy of endoscopic subureteric injection of autologous peripheral blood cells along with platelet-rich plasma. **Methods:** This clinical trial study was performed in the Urology department of Imam Reza Hospital in Mashhad, 2015. A total of 17 vesicoureteral reflux patients, 15–35 years old, who fulfilled our inclusion criteria, were evaluated. The endoscopic injection was administered by one surgeon in all patients at 6 o'clock position and 0.5 cm beneath the ureteral orifice, followed by renal ultrasonography and voiding cystourethrogram (VCUG), 3 and 18 months after the injection. The success of the injection was determined according to the disappearance of the symptoms and remission of the vesicoureteral reflux in following VCUGs. If the reflux persisted, the injection was re-administered after three months. **Results:** The success rates of each unit after the first and second injections were 89% and 94%, respectively. All the patients were evaluated by ultrasonography and VCUG for any reflux recurrence. Complications such as late recurrence and obstruction during 18 months follow-up were not determined. **Conclusion:** This study suggests that endoscopic injection of total blood nucleated cells and platelets is effective for the treatment of vesicoureteral reflux and recurrent pyelonephritis. This endoscopic injection is simple, non-invasive, non-antigenic, and with an acceptable success rate.

Keywords: Vesico-Ureteral Reflux, blood platelets, cell- and tissue-based therapy, injections, treatment outcome

INTRODUCTION

Vesicoureteral reflux (VUR) is a common disease characterized by a retrograde current of urine from the bladder toward the kidneys [1-3]. Its prevalence among adults is about 17%, also associated with several complications, like urinary tract infection, abnormal dilation of ureters, and underdeveloped kidneys (or dysplasia) [4]. There is a recent increase in the prevalence rate due to a higher rate of prenatal hydronephrosis detection rate [1-3, 5].

The spontaneous improvement of VUR, which is expected in children, is generally not observed in adults [6]. Persistent VUR leads to kidney scarring and reflux nephropathy, which ultimately leads to flank pain, hypertension, proteinuria, and, eventually, kidney failure. Thus, appropriate treatment of VUR is mandatory to prevent adverse clinical outcomes and control the morbidity VUR [7]. Medical management is long-term prophylactic antimicrobial therapy to prevent pyelonephritis.

Traditionally, if pharmacological treatment fails, the only alternative would be ureteral reimplantation surgery [8]. Since 1981, the efficacy of subureteric injections of bulking substances proposed as a treatment option for VUR [9]. For our purpose, we need a volume-stable agent with minimal allergic components. It also has to be safe, injectable, with the

Address for correspondence: Salman, Soltani (MD), Urology department, Imam Reza hospital, Mashhad, Iran.
Email: SoltaniS@mums.ac.ir

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least capacity of migration from the injection site^[10]. Several substances have been proposed for endoscopic injection within the subureteric region. Autologous agents like fat, collagen, and chondrocytes are particularly favorable because they do not incite an immune response. Polytetrafluoroethylene, polydimethylsiloxane are examples of non-autologous; however, the gold standard of the bulking agent is Dextranomer/hyaluronic acid copolymer (Deflux®). This study aimed to evaluate the therapeutic efficacy of total blood nucleated cells and platelets injection in vesicoureteral reflux. The goal of this study is to determine whether autologous blood nucleated cells would have the same success rate as non- autologous materials.

MATERIALS AND METHODS

This clinical trial was performed after approval of the ethical committee (code: 921671) in the Department of Urology of Imam Reza Hospital in Mashhad, 2015.

According to the sample size formula in the study of Okeke *et al.*^[11], 17 patients were enrolled in the study. Seventeen women with VUR were documented by voiding cystourethrogram (VCUG) and fulfilled our inclusion criteria included in the study. The inclusion criteria were a diagnosis of VUR by a recent VCUG, age >18 years, recurrent pyelonephritis, and a clinical history of VUR. The age of the patients ranged from 18–35 years (childbearing age). Two of the 17 patients had bilateral VUR hence a total of 19 injections performed. The total blood nucleated cells and platelets processed from the peripheral blood cells in the laboratory were used as the bulking agent. VUR was graded as Grade II in 6, Grade III in 10, and Grade IV in 3 units. Cortical scars were observed by dimercaptosuccinic acid scanning (DMSA) in 11 out of 17 renal-units.

Each injection contained whole blood cells with platelet growth factors derived from the peripheral blood of the patient. The patient was admitted one day before injection, and a 50 mL blood sample was collected from the patient. The collected blood was sent to specialized laboratories for the isolation of peripheral blood cells and platelet growth factor preparation. Platelet growth factors were prepared as follows: at first, the blood samples were centrifuged at 2000g for 8 minutes for sedimentation of RBC. Then, the obtained platelet-rich plasma is centrifuged at 4000g for 15 minutes to pellet the platelets. After removing the supernatant, 1 ml of platelet concentrate containing platelet-derived growth factors was obtained. This laboratory method was based on clinical-grade hydroxyethyl starch cells. 4 ml of cell suspension was added to the concentrated platelets isolated from peripheral blood in a sterile condition so that the total volume is 5 ml and transferred at 4° C to the urology department.

Injection for all patients was performed by one surgeon. 19 Fr cystoscope with 3–5 F ureteral catheters and an 18–23 gauge needle was used. The injection was made at 6 o'clock position and 0.5 cm beneath the ureteral orifice. All injections

were performed under general anesthesia, and the patients were discharged on the day of surgery. The mean volume of the bulking agent was 1 ml (range 0.5–1.8 ml according to the reflux grade). The patients were followed up by VCUG and ultrasonography 3 and 18 months later. The success rate was determined as the absence of VUR in post-operative imaging and the disappearance of the clinical symptoms. Lack of improvement after the first injection, followed by another injection three months later.

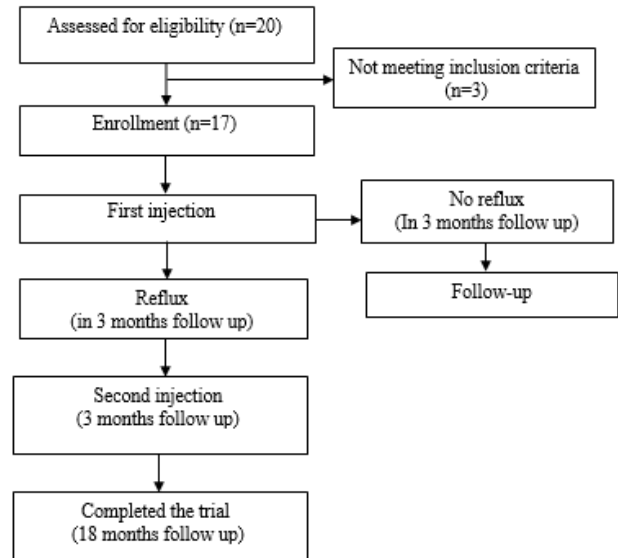


Figure 1-The flow-chart of trial

Statistical analysis

Data were imported to SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 16.0. Armonk, NY: IBM Corp) software and analyzed in terms of descriptive statistics parameters such as frequency, percentage, mean, standard deviation, and were presented in the form of tables and graphs.

RESULT

VUR is a dangerous situation during pregnancy; therefore, the treatment before conception is essential. All patients in this study were women in the age range of 18–35 (childbearing age, mean 22.8 ± 4.08 years; Figure 2).

Among the 19 renal-units studied, reflux grade distribution was as follows: Grade II: 6 renal-units, Grade III: 10 renal-units, Grade IV: 3 renal-units (Table 1).

Among the total 19 renal-units, the first injection was successful in 17 renal-units (89% success). Two renal-units (Grade IV) failed to respond to the first injection. After the first injection, lower grade refluxes (Grades II and III) had a better response to treatment than reflux in higher grades. Only 1 out of 3 of Grade IV showed a complete response to treatment (Figure 3).

The second injection was administered three months later in 2 patients. One patient had a complete response (66% of Grade IV after the first and second injection). In the third patient, the grade of reflux decreased from IV to II (Figure 4-5).

The complications, including non-febrile urinary tract infection or pain, were observed in six patients, but these complications had been resolved after medical treatment. Other complications such as major and minor ureteral obstruction, bulking agent migration, embolism, and febrile urinary tract infection were not observed. None of the patients showed an allergic reaction (Table 1). Our results showed that by increasing the frequency of injection, reflux grade could be reduced (even in the absence of full improvement of reflux). All patients were evaluated using ultrasonography and cystography for any reflux recurrence. We did not encounter any late recurrence or obstruction during 18 months follow-up.

DISCUSSION

In our study, the total blood nucleated cells and platelets were injected in 19 renal-units that showed an 89% success rate in the first injection and 94% success rate after the second injection assessed during a follow up of 18 months. The decreased success rate was obvious in the first injection when Grade IV reflux was considered (Grade IV reflux needs more injection).

Chertin and colleagues injected polytetrafluoroethylene to 717 ureters with high-grade reflux (IV- V) in pediatric patients. 58% of ureters were treated after the first injection and 26% resolved after second and third injections—17% improved reflux to grades I and II. A follow-up of 14 years showed a 98% resolution of VUR. Unlike our study, the outcomes were the same between grades IV and V. However, due to the risk of migration, the polytetrafluoroethylene is not currently used [12].

Reunane and colleagues performed collagen injections in 197 ureters with grades III and IV of VUR. For simple ureters, after one month, six months, two years, and four years of follow-up, VUR resolution was 93.9%, 91.7%, 85.3%, and 81.8%, respectively. These measures for patients with duplex ureters 44.4%, 25.9%, 23.1% and 21.4%, respectively [7]. Haferkamp performed only one injection of collagen in 58 ureters. After three years of follow-up, only 9% remained reflux-free—reported results for the injection of collagen [6]. The lower success rate in comparison to the Reunane study is probably due to the different number of injections and mispositioning of injections, as described in Haferkamp's study.

Polydimethylsiloxane is another material used for reflux treatment [13-16]. The size of this material is usually more than 100 μm , but the existence of some particles smaller than 80 μm can increase the risk of migration [17].

Atala and his colleagues used autologous chondrocyte in the treatment of animals with reflux [18]. However, the proposed method is not used nowadays due to the two anesthesia procedures, one for the cultivation of cartilage cells and prepares it for injection and another for the endoscopic injection. Moreover, its durability after a 1-year follow-up is under question.

Calcium hydroxyapatite (Coaptite) received FDA approval in 1998 for use in stress incontinence. It was used in 10 centers in the United States for the treatment of VUR [19] and showed a success rate of 46% at the end of 1st year and 40% at the end of a 2-years follow-up.

Another agent for endoscopic treatment of VUR is dextranomer/hyaluronic acid copolymer (Deflux). It has a short-term efficacy of 68-92%, and several studies showed favorable long-term outcomes, either [12, 20, 21].

For example, Kirsch used the hydro-distention implantation technique (HIT) and reported that short-term results of Deflux are similar to open surgery [22].

Lee and colleagues [23] indicated a relatively high failure rate in pediatric patients by using subureteric transurethral injection (STING). Of the total 337 ureters, 246 (73%) of ureteral units showed immediate resolution (no signs of VUR on VCUG after 6-12 weeks). In the long-term follow-up of primary responded patients, 73% remained reflux-free. The overall failure rate after one year was 53.9%.

Comparing our study with those who used non-autologous materials as the bulking agent, we found a similar success rate with fewer complications [22, 24, 25]. A recent meta-analysis in 2016 proposed that HIT is preferred over the STING procedure regarding its promising outcomes [26].

A meta-analysis by Elder *et al.* reported a success rate of 78.5% for reflux Grades I and II, 72% for Grade III, 63% for Grade IV, and 51% for Grade V after three months of follow-up [27].

The tissue-engineered lyophilized prepuce is proposed recently to be used as a bulking agent with satisfying results in animals [28].

In this study, the absence of symptoms after the sub-ureteral injection suggests that these patients may benefit from repeated endoscopic treatment. One of the main benefits of this method is the easy extraction of autologous non-invasive material from 10 mL of peripheral blood, which can be processed within a short time while other autologous materials require biopsies from various tissues of the body under anesthesia and a long period of reprocessing and re-injection with another anesthesia. Significant advantages include low-cost material compared with Deflux (75% cheaper than Deflux). Another benefit of this material is non-antigenicity.

A limitation of our study was the small number of patients and shorter follow-up time. Hence, it is suggested that this study should be repeated with more samples and longer follow-up to determine the durability of the material. Besides, the study recommends including patients from both genders in childhood with higher grades of reflux.

CONCLUSION:

This study suggests that endoscopic injection of total blood nucleated cells is an effective treatment method for vesicoureteral reflux and recurrent pyelonephritis. The endoscopic injection of total blood nucleated cells is simple, non-invasive, and non-antigenic with an acceptable success rate.

Ethical issues

All participants were provided with information about the study, both verbally and by written informed consent. All those who had the exclusion criteria, including those who preferred not to attend at any stage, were withdrawn from the study. Each patient gave informed written consent to participate in the study, which was approved by the Ethics Committee of Mashhad University of Medical Sciences. This form was agreed upon and completed by all subjects. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Conflict of interest

None.

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Table 1: Primary grade and complications after treatment of patients with vesicoureteralreflux

Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Flank pain	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-
Ureteral obstruction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cystitis	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-
Pyelonephritis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Migration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Primary grade	3	2	3	4	3	3	2	3	3	2	2	4	3	2	4,3	3,3	2

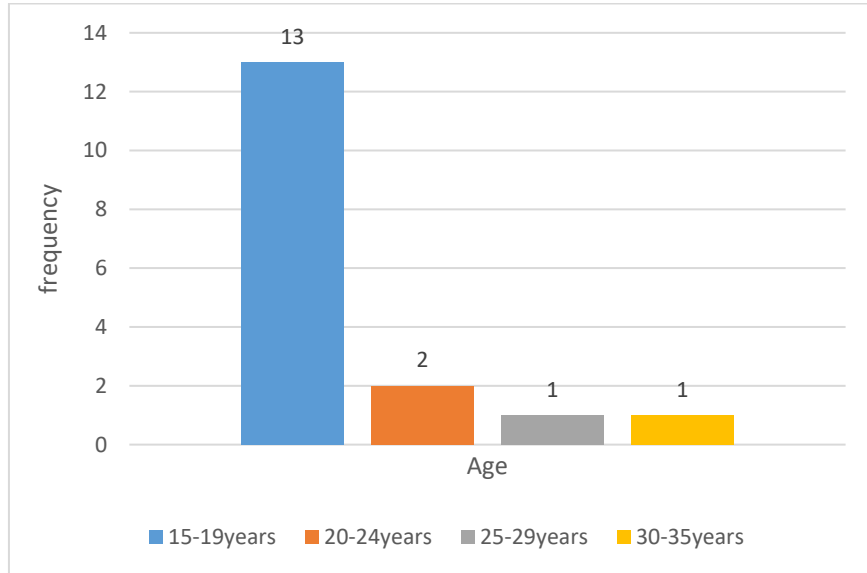


Figure 2: Age distribution of patients with vesicoureteral reflux



Figure 3: comparison between the complete responses after the first injection based on grade in patients with vesicoureteral reflux



Figure 4: comparison between the complete responses after the second injection based on grade in patients with vesicoureteral reflux

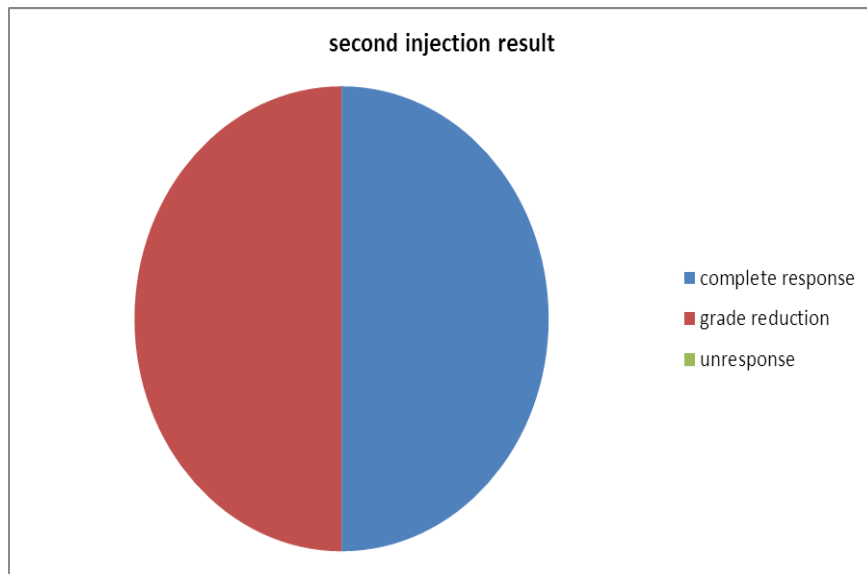


Figure 5: percent of response after second injection in grade 4