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Vitamin A supplementation is effective for improving the clinical symptoms of urinary tract infections and reducing renal scarring in girls with acute pyelonephritis: a randomized, double-blind placebo-controlled, clinical trial study

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ABSTRACT

Introduction: It is believed that tubulointerstitial inflammation plays a role in the formation of renal scarring secondary to acute pyelonephritis (APN). Vitamin A is an anti-inflammatory agent that is involved in the re-epithelialization of damaged mucosal surfaces.
Objective: The aim of this study was to evaluate the efficacy of vitamin A supplementation in combination with antibiotics for improving urinary tract infections (UTIs) symptoms and preventing renal scarring in girls with APN.
Study design: This randomized, double-blind, placebo-controlled clinical trial was conducted on 90 girls aged 2 to 12 years old between 2015 and 2017. Patients with UTIs and first episode of APN diagnosed based on 99 mTc-DMSA scintigraphy (uptake defect) were assessed for eligibility. Patients were randomly divided into two groups that either received 10 days of oral vitamin A (intervention group) or 10 days of placebo (control group) in addition to antibiotics during the acute phase of infection. The clinical response was considered as the primary outcome [duration (positive days) of UTI symptoms during trial treatment period] and secondary outcomes (no change, improving and or worsening of 99 mTc-DMSA scan results 6 months after treatment from baseline). $P < 0.05$ was considered to be statistically significant.
Results: Seventy-four patients (vitamin A group: 36 patients, placebo: 38 patients) were included in the analysis. The mean age was 5.25 ± 1 year old. Three patients (7.89%) in the placebo group and 2 patients (5.55%) in the vitamin A group had vesicoureteral reflux (VUR) ($p = 0.114$). Duration of fever (vitamin A group: 1.8 days, placebo: 3.1 days, $p = 0.0026$), urinary frequency (1.3 days vs. 2.8 days, $p = 0.003$) and poor feeding (2.3 days vs. 4.2 days, $p = 0.005$) were significantly lower in the vitamin A group. Following the second 99 mTc-DMSA scan, worsening of lesions was observed among 8 (22.2%) and 17 (44.7%) patients in the vitamin A and placebo groups, respectively ($p = 0.003$). 63.8% (23 patients) of the vitamin A group and 21% (8 patients) of placebo group showed lesion improving in the photopenic region. ($P < 0.0001$) There was no evidence of vitamin A intolerance.
Discussion: Our results show the efficacy of vitamin A supplementation on reducing renal scarring secondary to APN and on fever, urinary frequency and poor feeding duration in girls with APN.
Conclusion: Vitamin A supplementation is effective for improving the clinical symptoms of UTI and reducing renal injury and scarring following APN in girls with first APN. However, larger randomized clinical trials (RCTs) with longer follow up are needed to confirm these effects.

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1. Introduction

Urinary tract infection (UTIs) is considered as one of the most common infections of childhood and also the most frequent infection of the urogenital system. [1]

Risk factors for renal parenchymal damage in UTIs are the presence of vesicoureteral reflux (VUR),[2] obstructive uropathy, the numbers of flares of acute pyelonephritis (APN), and delay in treatment of acute infection.[3–6]

It has been shown that the incidence of renal scarring following APN ranges from 5 to 57% [7,8] and is associated with increased risk of progressive renal damage. In the worst cases, renal scarring after APN in children may lead to long-term cardiovascular morbidity and chronic kidney failure.[9] Severe renal scarring has also been reported after the first episode of APN.[10]

The pathogenesis of APN is related to bacterial virulence, immune response, tissue factors, apoptosis, tubulointerstitial inflammatory processes and the production of free radicals that lead to fibrosis and renal scarring.[11,12]

Antibiotic treatment is not always effective in preventing kidney scarring.[13] In a multicenter study, the early or late administration of antibiotics was shown to have no effect on the prevalence of renal scar formation in children with APN.[14]

Given the mechanisms underlying pyelonephritic renal damage, it can be speculated that anti-inflammatory or antioxidant therapy given concomitantly with antibiotics should lower the risk of post-pyelonephritic scarring[9] One promising strategy is therapy with vitamins as antagonists against free oxygen radicals and as anti-inflammatory agent. Results from animal studies have provided some evidence to back up this notion,[15–17] but research into the effects of vitamin therapy in humans has been surprisingly scant and controversial.[9]

Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, retinoic acid, and several provitamin A carotenoids (most notably beta-carotene).[18]

Vitamin A is an anti-inflammatory agent [18,19] Also, B-carotene was reported to be effective in inactivating free oxygen radicals.[20] Due to the role of retinol as a hormone-like growth factor for epithelial cells, Vitamin A also functions in the re-epithelialization of damaged mucosal surfaces[21–23] Kavukcu et al.[24] showed that the role of vitamin A in the re-epithelialization of damaged mucosal surfaces is one of the most important factors in preventing renal scarring and reducing the severity of the infection.

An early animal studies showed that the administration of vitamin A supplements in a rat model with UTIs reduces the incidence of renal scarring[22] and renal scarring is more severe when serum vitamin A levels are lower.[22,24] On the contrary, in another study, the administration of vitamin A not only failed to considerably affect the clinical and pathophysiological course of renal ablation nephropathy in rats but also when given at higher doses it could cause damage to kidney tissue.[25]

In human's studies on children, Dalirani et al[23] and Ayazi et al[26] in 2011, reported that vitamin A supplements were effective in reducing renal scarring secondary to APN.

According to meta-analysis by Zhang et al[9] in 2016, because of limited clinical studies and poor methodological quality of the past vitamin A trials on children with APN, which could significantly arouse a spurious result, high-quality and adequately powered RCTs with clearly defined end-points, are needed to further confirm of vitamin A benefits on children renal scaring.

Also so far, no clinical study has been conducted regarding the effects of vitamin A on both the acute phase (clinical symptoms) and the chronic complications (renal scarring) of UTIs.

The aim of this study was to evaluate the efficacy of vitamin A supplementation in combination with antibiotics for improving UTI symptoms and preventing renal scarring in girls with first APN.

Since UTIs are more common in girls due to their shorter urethras, and boys suffering from UTIs usually have underlying anatomical or functional abnormalities of the genitourinary tract and have a higher rate of primary scarring that would confound the study[27,28] we restricted our research to girls with pyelonephritis.

2. Materials and methods

2.1. Study design and randomisation

This was a randomized, double-blind, placebo-controlled clinical trial conducted from March 2014 until June 2017 in the Academic Pediatric Infectious Disease and Pediatric Nephrology departments of Amir-Kabir Hospital in Arak, Iran. Patients were randomly assigned to the intervention or control groups. Simple randomization using a computer-assisted randomization table was used for the current study.

2.2. Participants

The girls with UTI were evaluated for inclusion and exclusion criteria for recruit into the trial.

The inclusion criteria were aged between 2 and 12 years old, first-time diagnosis of APN based on clinical, laboratory, and 99 mTc dimercaptosuccinic acid (DMSA) scintigraphy renal scan confirmed evidence of pyelonephritis (reduced focal or multifocal perfusion defects in one or both kidneys), indications for hospitalization due to UTI, body temperature ≥38.5 °C, potty-trained and able to void volitionally. Since *E coli* is the most common cause of UTI and for easier cloning of the subjects for UTI factor organism, only the patients with UTI resulted by *E coli* were included in the study.[3]

The exclusion criteria were a history of recurrent UTIs, normal baseline 99 mTc-DMSA scan, renal scarring on the baseline scan, critically ill condition including: frequent vomiting, severe dehydration, sepsis and etc., systemic hypertension, neurogenic bladder, obstructive uropathy, low baseline serum levels of vitamin A (< 30 μg/dl), VUR grade > III, urinary calculi, Dysfunctional voiding (DV) and any unilateral or bilateral renal anatomical anomaly based on ultrasonography and voiding cystourethrography (VCUG). Also, participants with the lack of response to IV ceftriaxone therapy; non-Ecoli UTI; non-cooperation or dissatisfaction with continued participation; oral vitamin A intolerance and recurrent UTI during follow-up period were excluded from the study.

Due to the onset of trial in the earliest possible time in the acute phase of infection and do not wait for U/C results 48–72 hours after treatment for excluded non-Ecoli UTI; we randomized all eligible patients and excluded non-Ecoli UTI during the follow-up duration. In this way, Ecoli UTI patients continued the study.

2.3. Screening

The indications for hospitalization included dehydration (moderate to severe), absence of a previous history of UTI, inability to enough drink fluids and take oral medications at least twice a day (based on pediatrician's opinion), vomiting, requirement for IV (intravenous) antibiotic therapy, and suspected concurrent blood infection and UTI[29] If dehydration was indications for hospitalization we excluded severe dehydration (*see inclusion/exclusion criteria*)

Dehydration was evaluated based on clinical judgment and attention to parental reports and clinical signs and symptoms such as the amount of food and intake fluids, and the fluid outflow from the body (History of vomiting, diarrhea or reduced fluid intake), capillary filling time, abnormality of skin turgor, respiratory pattern and etc.

DV will refer to daytime voiding disorders in children who do not have neurologic, anatomic, obstructive, or infectious abnormalities of the urinary tract. DV diagnosed according to medical history, physical examination, imaging studies (sonography and VCUG) and finally pediatrician's opinion.

APN was diagnosed based on a high-grade fever (> 38.5 °C) with or without vomiting, malaise, loin pain, costovertebral angle tenderness, the absence of other sources of fever on the clinical examination, and imaging findings.[28]

2.4. Ethical considerations

All parents were given clear explanations regarding the methodology of the research and the lack of any harmful effects due to the dosage of vitamin A that was administered. The study protocol was approved by the Ethical Committee of Arak University of Medical Sciences, and the code 4-144-92 was assigned. Written consent was obtained from all the patients' parents or guardians. The patients were also free to withdraw from the study at any time. The study was conducted in accordance with the Declaration of Helsinki. The trial was registered at the Iranian registry of clinical trials (www.irct.ir; Trial Id: 30265, registration number: IRCT20130726014170N3) prior to the study.

2.5. Intervention

The control group received antibiotic therapy plus a placebo (once daily) and the intervention group received antibiotics in combination with oral vitamin A supplementation (1,500 U/kg/day; a maximum of 50,000 U; 50,000 IU tablets; once daily). (24, 27)

Baseline antibiotic therapy, vitamin A and the placebo were administered for 10 days (during the acute phase of infection). Antibiotic therapy with IV ceftriaxone (50 mg/kg/d to 75 mg/kg/d; CO Raazak, Iran) continued up to IV treatment response and was followed by the administration of prophylactic treatment with cephalexin syrup (15 mg/kg; CO Raazak, Iran) after discharge, totally (IV + orally antibiotics) up to day 10.

The vitamin A supplement and the placebo were provided by a pharmaceutical company (CO Jaber Ibn Hayyan, Iran). Two preparations were delivered to the same bedside physician in glass containers with lids of similar shapes and sizes, without a name and with a code. The placebos were similar to vitamin A (both small tablets) with regard to their shape, color, and size. The parents and children, the pediatrician who referred the patients, physician assessing the outcomes and nurses, isotope center physicians and laboratory staff were blinded to the treatment allocation. It was decided that children who could not swallow vitamin A or placebo tablets should be dissolved in water or crushed.

2.6. Examinations and follow-up

At baseline, demographic and clinical information such as clinical symptoms of UTIs were recorded. Laboratory tests and imaging studies including urinalysis (U/A) urine culture (U/C), blood culture (B/C), complete blood count (CBC), C-reactive protein (CRP, qualitative), erythrocyte sedimentation rate (ESR), renal and pelvic ultrasonography, VCUG, and 99 mTc-DMSA were performed. Laboratory tests (U/C, etc.), sonography and VCUG were performed on the first day of hospitalization. DMSA scan was carried out during the first 2 days of hospitalization.

Since 99 mTc-DMSA scintigraphy is the gold standard method for the diagnosis and localization of acute pediatric pyelonephritis[30] all the girls who qualified for the study's initial assessments underwent this scan for the evaluation of APN.

The clinical response was considered as primary and secondary outcomes. The primary outcomes were the duration (positive days) of UTI symptoms during 10 days treatment.

The secondary outcomes were changes of photopenic lesions with or without volume loss on 99 mTc-DMSA scan during 6 months; intolerance to vitamin A; changes in serum levels of vitamin A; U/C results and lack of response to antibiotic therapy.

The follow-up included: daily evaluation of the clinical symptoms of UTIs and vitamin A side effects over 10 days; U/C performed at 48 h and 7–10 days after treatment; measurement of serum vitamin A levels at the end of the treatment period (day 10), first and third months after treatment and a 99 mTc-DMSA scan at 6 and 12 months after treatment. (12 months after treatment scan was part of the plan but not carried out, *see Limitations section*)

At least one diagnosed episode per day was considered positive days for UTI symptoms including: Fever, vomiting, dysuria, diarrhea, frequency, urgency, dribbling, urinary incontinence, poor feeding and abdominal pain.

Bedside physician and nurses checked all the UTI symptoms daily by a questionnaire. During hospitalization, fever (> 38.5 °C) was checked 6 times a day (every 4 h) and other symptoms were checked daily based on subjectively (patients and/or parents) reported, nursing report and physical examination. Poor feeding was defined as Daily nutrition less than 50% of normal (before infection onset). Poor feeding was diagnosed on the basis of parental reports and nourishment monitoring during admission.

According to Yilmaz,[27] measuring serum levels of vitamin A 1 and 3 months after prescribing results in better evaluation.

Lack of response to antibiotic therapy was defined as unbreakable fever and no eradication on U/C, 48–72 hours after treatment.

The intolerance of vitamin A was defined as occurred following side effects and so according to pediatrician's opinion, the patient was not able to continue trial.

Vitamin A side effects including: vomiting; irritability; change skin color to yellow-orange; anorexia; dryness and scaling of the skin and lips that were monitored by history and clinical examination. Side effects were assessed daily in the 10-day treatment period.

After discharge, parents were warned about the vitamin A side effects and were emphasized to go to a medical center in case of any abnormal complication.

As long as the patients were admitted, the questionnaire was completed daily by physician and nurses. After discharge, it was emphasized to the parents that daily for 10 days, refer to evaluate clinical symptoms, side effects of vitamin A and U/C.

A midstream clean-catch urine specimen was used for U/C[3] For this method, the genital area was washed from front to back with soap and water 3 times, and intermediate urine samples were collected in sterile bags and transferred to the hospital laboratory. Mostly (especially in younger children) urine specimens provided by parents. Urine samples with infected medium were excluded.[3] Contamination of the culture medium was defined as a positive U/C without pyuria. A positive U/C was defined as the isolation of > 10^5 colony forming units (cfu)/ml of a single bacterium (*E. coli*) from a midstream urine sample[28]

The serum levels of vitamin A were measured according to the spectrophotometric Neeld and Pearson method[27] The normal range of serum vitamin A levels was 30–65 μg/dL.

2.7. mTc dimercaptosuccinic acid scintigraphy

Three to four hours following the IV injection of 37 MBq of 99 mTc-DMSA, several projection images (posterior, anterior, right posterior oblique, and left posterior oblique) were taken. Any photopenic lesion (small vs. large, unilateral vs. bilateral, single vs. multiple) that was observable on the posterior view without volume loss was defined as APN. The lesions were divided based on renal involvement into three groups, namely, mild (25%), moderate (25–50%), and severe (< 50%).[28] Scan results (second scan from first) were described as no change, improving or worsening.[29,31] for example, change from mild (first scan) to moderate (second scan), and etc. to be categorized as a worsening change and patient with same lesions in first and second scans was not to be categorized as a change.

The results of the renal scan were reported in the form of patient units, not kidney units. All the renal scans were secondarily reviewed by 2 independent nuclear medicine experts.

Different severities of renal involvement and worsening of lesions (increased involvement) were defined as the involvement of at least one kidney. Improving of lesions was defined as the reduction of involvement of both kidneys. A no change of 99 mTc-DMSA scan was defined as non-worsening or non-improving of both kidneys, respectively.

2.8. Statistical analysis

Sample size was calculated by using Altman nomogram with a power of 80%. For the estimation of sample size, results of other studies[24,27] regarding to the effect of vitamin A on our outcome were used. In addition, a significance level of 5%, and a power of 80% was assumed. The minimum sample size of 40 in each group was calculated and by assuming a 10% attrition rate, the final sample size of 45 was required in each group. Moreover, power calculations (i.e., to estimate the effect size) was checked by MedCalc software (MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium).

Statistical analysis was an On-treatment analysis. Therefore, only the patients completing the study entered the analysis. (Fig. 1)

The collected data were analyzed with Statistical Package for the Social Sciences (SPSS) software version 18.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics methods were used for frequency determination.

Continuously variable data with normal distribution are presented as mean ± SD, and Student's *t*-test was used to compare the means of the groups. If the data was not normally distributed, median with interquartile ranges is quoted and Mann–Whitney U tests were used to compare groups, respectively. Categorical variables were reported as *n* (%) and analyzed with Fisher's exact test and χ^2-test.

For serum vitamin A levels and positive U/Cs, one-way repeated measure analysis of variance (ANOVA) was used to compare the values among the follow-up steps.

linear regression analysis was performed to identify UTI symptoms positive days associated with vitamin A levels during 10 days' treatment by including all variables available at admission and during hospitalization as independent variables.

$P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Participants

Of the 193 potential participants who were approached, 111 (57.51%) patients (girls with UTI) assessed for eligibility, which among them 21 patients were excluded. Finally, 90 patients were recruited into the trial. We lost 16 patients during the follow-up period. Thirty-six patients were included in the vitamin A group, and 38 patients were included in the placebo group to complete the study. Fig. 1 illustrates the study recruitment process (CONSORT flowchart). Some patients were excluded due to withdrawal of consent between end of 10-day treatment and the repeat DMSA scan. Three patients (2 patients: Klebsiella, 1 patient: pseudomonas) in the vitamin A group and 2 patients (both of them due to Klebsiella) in the placebo group excluded due to non-Ecoli UTI. One patient in the vitamin A group did not respond to IV ceftriaxone therapy and was excluded from the study due to the need for another course of treatment. The remaining patients in the two groups were symptom-free during the 10-day course of treatment. All patients who completed the trial enable monitoring/observation during hospitalization and during discharge period up to day 10.

3.2. Demographic and baseline characteristics

The mean age of the participants was 5.25 ± 1 years old, (range: 3–12 years old). The mean age of the participants was 5.1 ± 2.6 years

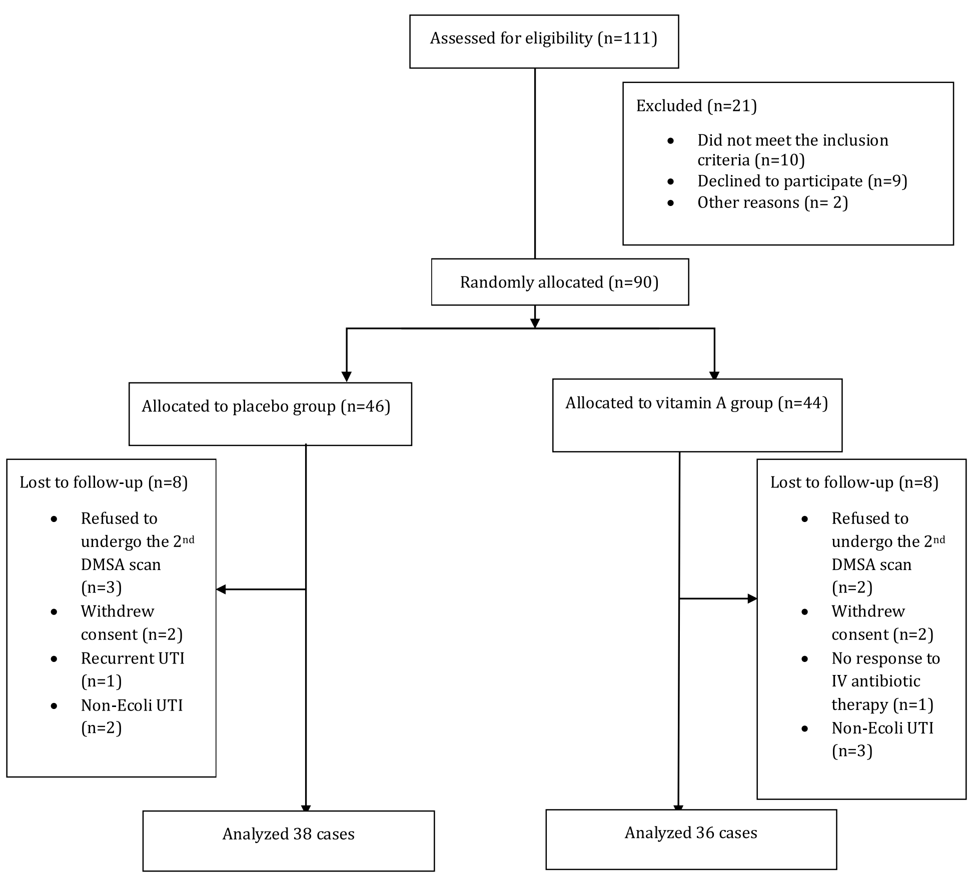

Fig. 1. CONSORT flowchart of patients.

Table 1
Demographic data and baseline characteristics.

Vitamin A group[b] (n = 36)	Placebo group[a] (n = 38)	Demographic and clinical findings
5.4 ± 2.1	5.1 ± 2.6	**Age (years)**
17.1 ± 3.8	16.9 ± 4.3	**Weight (kg)**
104.1 ± 15.2	106.4 ± 10.1	**Height (cm)**
1.4 ± 1.0	1.2 ± 0.4	**Time gap[c] (days)**
90 (75-110)	95 (70-105)	**SBP[d] (mmHg)**
60 (50-80)	60 (50-80)	**DBP[d] (mmHg)**
2 (5.5) (2 unilateral GII)	3 (7.8) (2 unilateral GII, 1 bilateral GII)	**VUR, n (%)**

DBP, diastolic blood pressure; SBP, Systolic blood pressure; VUR, vesicoureteral reflux; G, grade.
Data are presented as the mean ± standard deviation (SD).

[a] The placebo group received antibiotic therapy in combination with an oral placebo.

[b] The vitamin A group received antibiotic therapy in combination with oral vitamin A.

[c] Duration from the beginning of symptoms to hospitalization and the start of antibiotic therapy.

[d] analyzed with non-parametric statistic.

Table 2
Laboratory results at the beginning of hospitalization.

Laboratory results	Placebo group (n = 38)	Vitamin A group (n = 36)
WBC ($\times 10^3$)	13.4 ± 4.4	13.2 ± 8.1
PMN (%)	68.5 ± 5.2	70.8 ± 10.2
Hemoglobin (g/dL)	12.3 ± 6.2	12.5 ± 1.3
Creatinine (mg/dL)	0.5 ± 1.0	0.5 ± 0.3
BUN (mg/dL)	7.2 ± 3.2	6.9 ± 5.1
ESR (mm/h)	44.1 ± 12.3	49.8 ± 15.1
CRP, positive, n (%)	29(76.3)	30(83.3)
Pyuria (> 10/mm^3), n (%)	38(100)	36(100)
Nitrite-positive, n (%)	9(23.6)	8(22.2)
Bacteriuria, n (%)	38(100)	36(100)

WBC, white blood cell; PMN, polymorphonuclear leukocytes; BUN, blood urea nitrogen; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NS, not significant. Data are presented as the mean ± SD.

and 5.4 ± 2.1 years for the vitamin A and placebo groups, respectively ($P = 0.11$). All patients in the both groups at the beginning of hospitalization were febrile and the mean body temperature was 38.8 °C. The mean of hospital stay in all patients were 3.5 ± 1.2 days (range: 2–5 days). Both groups were matched for basic demographic and clinical features. (Table 1)

In the vitamin A group, 3 patients (7.89%) had unilateral or bilateral VUR grade II, and in the placebo group, 2 patients (5.55%) had unilateral or bilateral VUR grade II. No patients in either group had physical signs of malnutrition or vitamin A deficiency. The laboratory results at the time of admission are shown in Table 2. Forty-eight patients (64.86%) out of 74 patients had increased peripheral white blood cell (WBC) counts. There was no significant difference between the two groups in CRP positive numbers. [vit A group: 30 patients (83.3); placebo: 29 (76.3)]. All the patients had normal serum creatinine levels. Pyuria and bacteriuria were observed in all the patients on the initial urinalyses. Both groups were matched for laboratory results.

The frequencies of patients with baseline UTI symptoms at the beginning of hospitalization in the intervention and control group are presented in Table 3. Both groups were matched for all baseline UTI symptoms. The most frequent baseline symptoms in all girls (74 patients) were fever (100%), followed by poor feeding (41 patients, 55.4%), vomiting (21, 28.37%), urinary frequency (19, 25.67%), dysuria (15, 20.27%), abdominal pain (13, 17.56%), urgency of urination (12, 16.21%), incontinence (11, 14.86%), diarrhea (9, 12.16%) and dribbling urine (7, 9.45%).

Table 3
Frequencies of patients with baseline UTI symptoms in the two groups.

Symptoms[a]	Placebo group (n = 38)	Vitamin A (n = 36)	P value
Fever (> 38.5 °C), n (%)	38 (100)	36 (100)	0.177
Vomiting, n (%)	9 (23.6)	11 (30.5)	0.113
Diarrhea, n (%)	5 (13.1)	4 (11.1)	0.3
Frequency, n (%)	8 (21)	11 (30.5)	0.061
Urgency, n (%)	7 (18.4)	5 (13.8)	0.42
Dysuria, n (%)	9 (23.6)	6 (16.6)	0.62
Dribbling, n (%)	4 (10.5)	3 (8.3)	0.99
Urinary incontinence, n (%)	7 (18.4)	4 (11.1)	0.072
Poor feeding, n (%)	22 (53.6)	19 (52.7)	0.81
Abdominal pain, n (%)	5 (38.4)	8 (22.2)	0.51

[a] At the beginning of hospitalization.

3.3. Primary outcome

The duration (positive days) of UTI symptoms during 10 days treatment is shown in Fig. 2. The duration of fever (vitamin A: 1.8 days, placebo: 3.1, p = 0.0026), urinary frequency (1.3 vs. 2.8, p = 0.003) and poor feeding (2.3 vs. 4.2, p = 0.005) were significantly less in the vitamin A group than in the placebo group.

3.4. Secondary outcomes

3.4.1. Urine culture

The number of positive U/Cs was not significantly different between the two groups 2 days after treatment (vitamin A: 3 (8.33%), placebo: 4 (10.52%), p = 0.17) and 7–10 days after treatment (1 (2.77%) vs. 0, p = 0.31). Based on ANOVA, the number of positive U/Cs in both groups significantly decreased within 10 days (in the two steps of U/C, placebo: p = 0.021, vitamin A: p = 0.001). On repeated cultures, the organism was *E. coli* in all cases.

3.4.2. mTc-DMSA scan

The summary of the 99 mTc-DMSA scan evaluations is given in Fig. 3. The mean follow-up time or the mean second 99 mTc-DMSA scan for vitamin A and placebo groups was 6.39 and 6.14 months, respectively. (P = 0.112) Renal involvement following the first 99 mTc-DMSA scan was not significantly different between the two groups.

On the second 99 mTc-DMSA scan, 18 (50%) girls of the vitamin A group and 8 (21%) girls of placebo group had normal results. (p = 0.003) The frequencies of patients with moderate (p = 0.0001) and severe (p = 0.001) involvement in the vitamin A group [moderate: 2(5.5%), severe: 1(2.7%)] was significantly lower than placebo group [moderate: 12 (31.5%), severe: 3 (7.8%)].

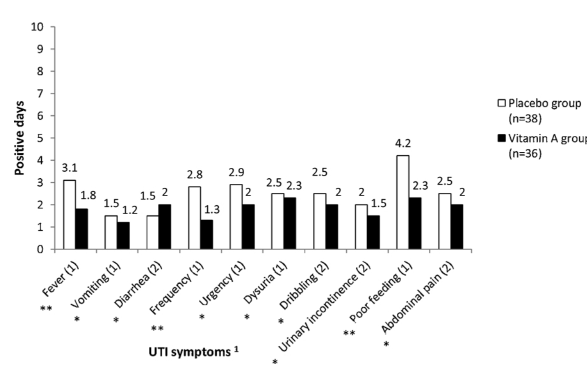

Fig. 2. The duration of UTI symptoms during 10 days treatment in each group. [1] At least one diagnosed episode of symptoms per day was considered positive days. Data are presented as the mean (1, Student's *t*-test) and median (2, Mann–Whitney U test) of UTI symptoms positive days. * $P < 0.05$, ** $P > 0.05$.

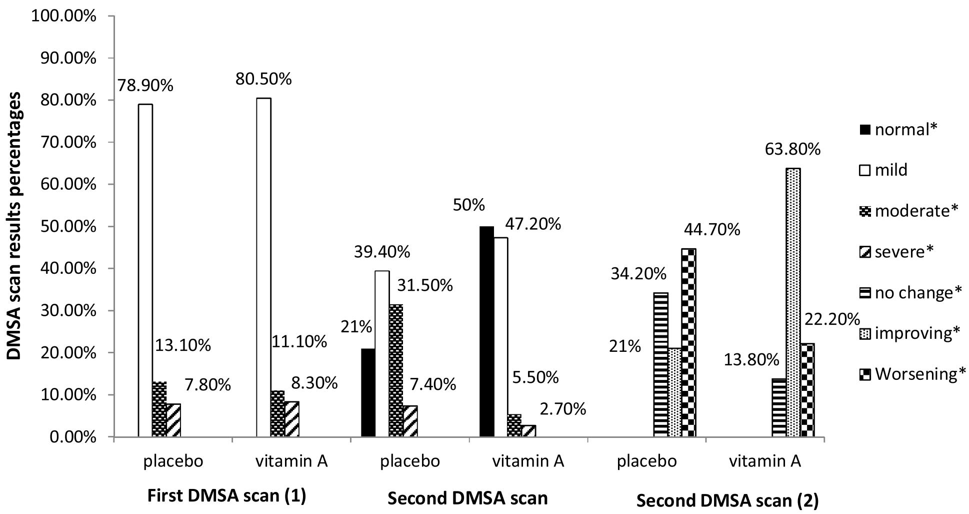

Fig. 3. Assessment of renal involvement based on the 99 mTc-DMSA scans lesions at the beginning of hospitalization (first scan) and at least 6 months later (second scan). DMSA, Dimercaptosuccinic acid scintigraphy. Data are presented as percentages of DMSA scan results[1] All grades indicate the acute pyelonephritis.[2] Changes in DMSA scan results: second scan from baseline. * Significant difference between vitamin A and placebo in second DMSA scan (normal: P = 0.003; moderate: P = 0.0001; severe: 0.001; no change: P = 0.04; improving: P < 0.0001; worsening: P = 0.003).

On the second 99 mTc-DMSA scan, 44.7% (17 patients) of the placebo patients showed lesion worsening in the photopenic region. In contrast, of the children who received vitamin A supplements, 22.2% (8 patients) showed evidence of worsening lesions at follow-up (p = 0.003). 63.8% (23 patients) of the vitamin A group showed lesion improving in the photopenic region. Lesion improving was shown in 21% (8 patients) of placebo group. (P < 0.0001) Photopenic lesions had not changed in 13.8% (5 patients) of placebo group and 34.2% (13 patients) of vitamin A group (p = 0.04)

3.4.3. Vitamin A levels

Baseline serum vitamin A levels were 56.2 ± 9.8 μg/ dL in the vitamin A group and 51.48 ± 18 μg/dL in the placebo group (p = 0.91). Serum levels of vitamin A in the first month (vitamin A: 69.8 ± 10.1 μg/dL, placebo: 49.1 ± 8.7 μg/dL, p = 0.008), end of 10 days treatment (65.13 ± 5.5 vs. 50.79 ± 11.1, p = 0.031) and in the third month (72.2 ± 7.2 vs. 53.1 ± 12.3, p = 0.02) were significantly higher in the vitamin A group.

In the vitamin A group, repeated-measure ANOVA showed significant increase levels of vitamin A at the end of treatment period (day 10) from baseline. (p = 0.0002) there was a significant increase in serum levels of vitamin A at the first month from baseline (p = 0.01) and 3rd month from baseline (p = 0.002). However, the changes during post treatment period [(first month from day 10: p = 0.441, 3rd month from first month: p = 0.6)]] were not significant. (Fig. 4)

In the placebo group, changes in the serum levels of vitamin A were not significant in none of the study periods related to vitamin A levels: Day 10 from baseline: p = 0.811; first month from day 10: p = 0.3; first month from baseline: p = 0. 112; 3rd month from baseline: p = 0.55; 3rd month from first month: p = 0.08)

Table 4
UTI symptoms associated with serum vitamin A levels over 10 days treatment period.

Symptoms[a]	P value[b]	Odds ratio	95 % Confidence interval
Fever	< 0.001	3.21	1.77-5.83
Frequency	0.022	4.14	1.85-9.32
Poor feeding	0.001	2.08	1.69-5.91

[a] UTI symptoms during 10 days treatment.
[b] based on linear regression analysis.

Based on linear regression analysis in the vitamin A group a significant relationship was observed between serum vitamin A level during 10 days treatment and duration (positive days) of fever (P < 0.001, OR = 3.21), urinary frequency (P = 0.022, OR = 4.14), and dysuria (P = 0.001, OR = 2.08) (Table 4).

In the placebo group, there was no significant relationship between serum vitamin A level and any symptoms of UTI during the 10 days of treatment.

There was no evidence of vitamin A intolerance. No adverse effects of vitamin A supplementation were reported during treatment.

4. Discussion

The results of this study show the efficacy of oral vitamin A supplementation during the acute phase of infection in resolving some of

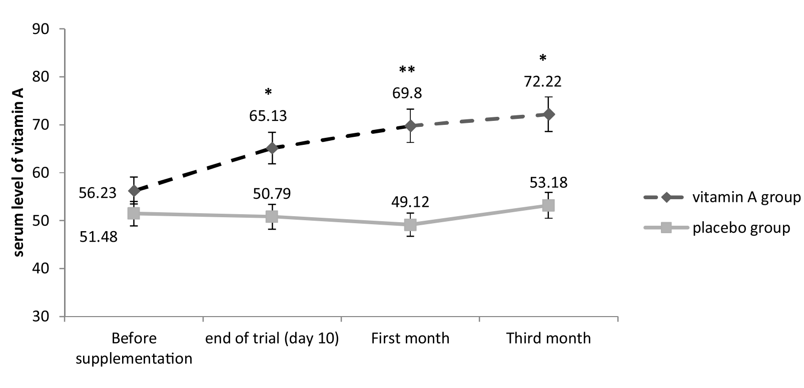

Fig. 4. Mean serum vitamin A levels during the follow-up periods in each group. * P < 0.05, ** p = 0.008.

Table 5
Comparison of the characteristics, design and results of present study with previous vitamin A trials on renal damage in children with APN.

variables	Present study	Dalirani/2011/[23]	Ayazi/2010/[26]	Sobouti/2012/[28]	Qin/2012/[33]
Country and study design	Iran; double-blind RCT	Iran; single-blind RCT	Iran; single-blind RCT	Iran; non-blind RCT	China; single-blind RCT
Age range and inclusion year of participants	24-144 months;2014-2017	2–144 months; 2007–2009	2–144 months; 2006–2008	Girls from 1–120 months; 2004–2006	24–72 months
Gender of participants	Only girls	Both genders	Both genders	Only girls	Both genders
No. of subjects in intervention/ control group (median or mean age in months)	36 (61.2)/ 38(64.8)	37 (16)/39 (12)	25 (24)/25 (24)	15 (42)/20 (18)	43 (44.4)/43 (44.4)
Intervention of controls and cases	Controls: antibiotics[a] Cases: additional vitamin A[b]	Controls: antibiotics[c] Cases: Additional vitamin A[d]	Controls: antibiotics[e] Cases: additional vitamin A[f]	Controls: antibiotics[g] Cases: additional vitamin A[b]	Controls: antibiotics[h] Cases: additional vitamin A[i]
No. of patients or kidney units with renal damage[j] (renal scaring or worsening lesion[k])	Vitamin A: 8/36; Control: 17/38	Vitamin A: 20/37; Control: 35/39	Vitamin A: 5/25; Control: 17/25	Vitamin A: 7/30; Control: 17/40	Vitamin A: 22/43; Control: 38/43
99 mTc-DMSA scan times	Baseline and after 6 months	Baseline and after 3 months	Baseline and after 6 months	Baseline and after 6 months	Baseline and after 6 months
UTI symptoms follow up period	Daily check-up for 10-day treatment	Not reviewed	Not reviewed	Only baseline	Not reviewed
Urine culture follow up period	At Baseline; 48 hours and 7-10 days after treatment	Not reviewed	Only at baseline	Baseline; after 3 days of treatment and then monthly to 6 months	Not reviewed
Serum vitamin A level measurement	Baseline; first month; third months	Only at baseline	Not reviewed	Not reviewed	Not reviewed

RCT, Randomized controlled trial; 99 m Tc-DMSA, technetium-99 m dimercaptosuccinic acid renal scintigraphy; Baseline, before starting treatment.
[a] intravenous ceftriaxone 50–75 mg/kg/d up to IV treatment response, followed by cephalexin syrup 15 mg/kg after discharge, totally (IV + orally antibiotics) up to day 10.
[b] Vitamin A 1500 U/kg/day orally for 10 days, with a maximum amount of 50,000 U daily.
[c] Ceftriaxone 75 mg/kg for 10 days intravenously.
[d] Vitamin A at 25,000 U for < 1 year and 50,000 U for ≥1 year, during the first 3 days intramuscularly.
[e] Ceftriaxone 75 mg/kg twice a day for 10 days intravenously, followed by cephalexin syrup (15 mg/kg) for 3 months.
[f] Vitamin A at 25,000 U for < 1 year and 50,000 U for ≥1 year, intramuscularly at the time of hospitalization.
[g] Cephalothin or ampicillin (100 mg/kg/day 4 times daily) and amikacin (15 mg/kg/day 3 times daily) for 10 days.
[h] Ceftriaxone 75 mg/kg twice a day for 10 days, followed by cephalexin syrup (15 mg/kg).
[i] Vitamin A at 1500 U/kg/day intramuscularly, with a maximum of 50,000 U daily for 10 days.
[j] only in study by Sobouti et al[27] renal damage reported as numbers of kidney units and not patient unit.
[k] No. of patients or kidney units with renal damage (renal scaring) in the present study and study by Sobouti[27] reported as worsening lesion.

the clinical symptoms of UTI more quickly and in reducing renal scarring secondary to APN. On the other hand, children who were treated only with antibiotics had a longer duration of symptoms and higher rates of renal scarring.

Side effects and inadequate response of antibiotic therapy in some cases; the high cost of IV therapy and hospitalization, and long-term and destructive complications of renal scar formation, Side effects and inadequate response of antibiotic therapy in some cases; the high cost of IV therapy and hospitalization, and long-term and destructive complications of renal scar formation.[3,26,28]

The role of vitamins, non-steroidal anti-inflammatory drugs (NSAIDs), and steroids in reducing renal cortical scar formation after APN has been studied experimentally.[3,25,28] However, human' data is limited.[9]

Due to the dominant pathophysiology-tubulointerstitial inflammatory processes and oxidative stress -in renal scar formation; changes of vitamin A levels in the body during infections[28,32]; supportive findings of animal studies,[22,24,25] and known anti-inflammatory and anti-oxidative roles, vitamin A was recognized as a potential therapeutic target for reducing of renal damage in children with APN.

In the present study, of 36 patients with primary abnormal renal DMSA scan in the vitamin A group, only eight (22.2%) of children were demonstrated to have worsening of lesions on their second scans 6 months after the hospital discharge. Among the placebo group, the results of repeated DMSA scans were noticeable difference, so that seventeen (44.7%) of patients have worsening of lesions and the difference between two groups was shown to be statistically significant. Also, our patients who received supplements of vitamin A, despite having lower renal damage in the photopenic region on the repeated DMSA scan, had higher lesion improving (63.8%) compared to the placebo group (21%), on the second scan.

Dalirani et al[23] administered therapeutic dosage of vitamin A intramuscularly to 76 children and found a change in progression of kidney injury and scaring on the repeat 99 mTc-DMSA scan in favor of vitamin A administration compared to placebo after 6 month ($P < .001$). In a non-blind RCT by Sobouti et al[28] on children with APN, they found less worsening of lesions in the vitamin A group (23.3%) compared to controls (42.5%) ($LR = 26.3$, $P < 0.001$), after 6 months. Administration of vitamin A was associated with a significantly lower rate of permanent renal damage in a single-blind study by Ayazi et al[26] At the repeat DMSA scan after 3 months, they reported 20% of vitamin A group compared to 68% of control had abnormal findings ($p = 0.001$).

Zhang et al[9] in a meta-analysis evaluated four past vitamin A trials on the renal damage in children with APN, which are shown with details in Table 5. They were investigated 248 patients aged 1–144 months (120 in the intervention group, 128 in the control group). Results showed that vitamin A was inversely associated with renal damage (relative risk 0.53, 95% confidence interval 0.43-0.67) when compared with placebo after an average follow-up of 5 months.

Align with our hypothesis and the main conclusion, Dalirani,[23] Ayazi,[26] Qin[33] and also Sobouti[28] confirmed that administration of vitamin A is useful and effective in decreasing the amount of the injury and scarring following the pyelonephritis. Also, Zhang's meta-analysis main results were consistent with our hypothesis. Nevertheless, there are some differences in study characteristics, design, variables and details of the results between our study and past trials (Table 5).

Vitamin A administration in the present study and Sobouti's study[28] was orally daily for 10 days, while vitamin A was prescribed single dose intramuscularly in Ayazi's study[26] and Dalirani study.[23] Qin[33] administered intramuscularly vitamin A daily for 10 days.

In our repeated DMSA scan, 22.2% of patients in the vitamin A group had worsening of lesions compared to 44.7% of patients in the control group. The frequency of renal scarring in the second scan in the study by Ayazi[26] (vitamin A: 20%, control: 68%) and Sobouti[28] (vitamin A: 23.3%, control: 45.5%) was almost consistent with our study. However, in the second scan the frequency of renal damage or scaring in study by Dalirani[23] (vitamin A: 54%, control: 89.7%) and Qin[33] (vitamin A: 51.1%, control: 88.3%) was more of our study in both groups. The greater of renal damage frequency in repeated DMSA scan in studies by Dalirani[23] and Qin[33] relative to our findings can be due to single-blind design; the manner of baseline antibiotic treatment and vitamin A administrated, and DMSA scan follow-up duration.

In contrast to our design, Dalirani[23] performed a second DMSA scan at 3 months after the start of treatment. According to Jakobsson et al[29] the duration of follow-up can contribute to the development of the scar. Sobouti[28] found that the duration of 3 months after UTI is not a sufficient time for scar formation and that the repeated 99 mTc-DMSA scan should be delayed until least 6 months or longer to evaluate the establishment of the newly formed renal scars. In the present study, the repeated DMSA scan was done after 6 months. However, longer repeated scan duration could be more useful in interpreting the results.

Our study has some main advantages compared to past RCTs (Table 5) included: Double-blind design; used a placebo; investigation of clinical symptoms of UTI (daily for 10 days) and U/C follow up. Also, another advantage was measuring serum level of vitamin A. We believe that measuring serum level of vitamin A and assessing its connection with clinical symptoms of UTI can be a guide for future studies to examine the role of vitamin A in the incidence, duration and severity of UTI symptoms.

In vitamin A group, fever, frequency and poor feeding were significantly correlated with serum level of vitamin A during 10 days treatment. Following evidences of our findings can be explained communication of the faster improvement of fever and frequency with the increase of vitamin A serum level: 1) the significant lower of fever and frequency duration (positive days) in the vitamin A group compared to placebo; 2) OR > 1of fever and frequency in association with serum level of vitamin A (3.21 and 4.14, respectively) and 3) the significant increase in serum level of vitamin A alongside of fever and frequency reducing during 10 days relative to the baseline. Although serum level of vitamin A was correlated to poor feeding, the evidences are not enough to come to a conclusion regarding the effect of increase of vitamin A level over 10 days on poor feeding.

So far, there has been no evidence of antipyretic and anti-urinary frequency effects of vitamin A, and present study is the first report. These effects could be the result of anti-inflammatory role of vitamin A, yet more studies are needed for more comprehensive conclusions.

In the present study, taking vitamin A supplementation had no side effects. Previous vitamin A trials on children (Table 5), also did not report any side effects or intolerance to either oral, IV or IM injectable vitamin A.

4.1. Limitations

First, the 99 mTc-DMSA scan follow-ups were scheduled to be performed in three steps (at the beginning of the study, and 6 months and 12 months after treatment). However, due to lack of cooperation from the patients' parents and the lack of necessary equipment, a third scan was not carried out.

Other limitations of this study were limited generalizability given the strict eligibility criteria (small age range, only females, only Ecoli UTIs); lack of adjustment for other potential unmeasured confounders, such as history of bladder/bowel dysfunction and on-treatment analysis.

Also potential selection bias given the relatively high drop-out rate (18% in the vitamin A group and 17.3% in the placebo) was one of the limitations. High drop-out rate mostly due to some patients not undergoing the second 99 mTc-DMSA scan; their unwillingness to continue to participate in the study and lack of baseline period.

The pediatricians' idea was to include only E. coli UTI for better matching of the patients. Due to the onset of trial in the earliest possible time in the acute phase of infection and do not wait for U/C results 48–72 hours after treatment for excluded non-Ecoli UTI, we did not have baseline period after infection diagnosis. In this way, drop-out occurred during the follow up due to non-Ecoli UTIs which increased the drop-outs. Therefore, we recommend future studies being conducted on patients with UTI excluding restriction of pathogens.

In this study, prophylactic antibiotics were prescribed to patients after discharge, up to day 10. According to some evidences, antibiotic prophylaxis could impact the development of a subsequent UTI. This decision was based on past clinical experience and opinion of the pediatricians. Also, in the study by Ayazi,[26] and Qin[33] cephalexin syrup (15 mg/kg) was performed as antibiotic prophylaxis after intravenous administration.

We reported no evidence of vitamin A intolerance and adverse effects, however the conclusion cannot state that Vitamin A supplement is safe - because this is not a safety study and it is not powered to examine safety.

Comparison of CRP normalization time between the two groups could have been of the useful results of the study. Due to CRP changes were not among the variables of primary and secondary outcomes, the comparison of its normalization time during the follow up between the two groups was not available in analyzing.

5. Conclusion

Vitamin A supplementation is effective for improving the clinical symptoms of UTI and reducing renal injury and scarring following APN in girls with first APN. Previous positive clinical trials evidence,[23,26,28,33] and meta-analyses with promising results,[9] can support our results. However, confirmation of the benefits of vitamin A supplementation is reducing renal scar formation in acute pyelonephritis needs larger randomized clinical trials with longer follow up period.

Authors' contributions

All the authors have contributed to drafting and revising the manuscript, study concept, and design, as well as data gathering and interpretation. All the authors reviewed and approved the final version of the manuscript.

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Conflict of interest

All authors declared that they had no conflict of interest.

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