Author's response to reviews

Title: Does Pomegranate intake attenuate cardiovascular risk factors in hemodialysis patients? Results of a randomized placebo controlled trial

Authors:

Lilach Shema-Didi (lilach.shema-didi@naharia.health.gov.il)
Batya Kristal (Batya.Kristal@naharia.health.gov.il)
Shifra Sela (Shifra.Sela@naharia.health.gov.il)
Ronit Geron (Ronit.Geron@naharia.health.gov.il)
Liora Ore (liora.ore@gmail.com)

Version: 4
Date: 16 December 2013

Author's response to reviews: see over
Title: “Does Pomegranate intake attenuate cardiovascular risk factors in hemodialysis patients? Results of a randomized placebo controlled trial” revised paper.

Authors:
Shema – Didi Lilach (lilach.shema-didi@naharia.health.gov.il)
Kristal Batya (Batya.Kristal@naharia.health.gov.il)
Sela Shifra (Shifra.Sela@naharia.health.gov.il)
Geron Ronit (Ronit.Geron@naharia.health.gov.il)
Liora Ore (liora.ore@gmail.com)

Version: 2 Date: 15 December 2013

Author’s response to reviews:
Reviewer 1:
1. Minor Essential Revisions
1.1. Comment: "In the sentence: “As well as to inhibit the angiotensin converting enzyme (ACE), a key component in the renin angiotensin aldosterone system (RAAS) which regulates blood pressure” the reference is missing."
Response: We add a reference.

1.2. Comment: "At the beginning of the conclusion, authors talk about the present study but they referenced a paper “13”. Is this a mistake? Please, they should to check"
Response: Reference 13 refer to the PJ properties ("PJ with anti-atherogenicity properties") and not to the study results.

1.3. Comment: In conclusions, bibliography shouldn’t appear
Response: We deleted the reference.

1.4. Comment: Figures don't have legend. The authors should add the legend to both figures

1.5. Comment: Discretionary Revisions Page 4. Just a curiosity: Is pregnancy common in this patients?
Response: Pregnancy in dialysis patients is not common but could be occur.

1.6. Comment: "Do the authors know the quantity of Vitamin C that the PJ juice have? Have they studied the influence of vitamin C on the lipid profile? Due to PJ juice is a fruit that could have a lot of vitamin C, the authors should mention something related to lipoperoxidation and vitamin C: Ramos R, Martínez-Castelao A. Metabolism. 2008"

Response: We didn't examine the quantity of Vitamin C. In the current study we did not focus on the PJ components but to the beneficial effect of the whole PJ juice.

Reviewer 2:

1. Reviewer report:

1.1. Comment: "It seems not to have been made a sample size calculation. It may be important since the authors published other article with the same cohort of patients (reference number 18) investigating the effects of PJ on oxidative stress, inflammation and also evaluating the incidence of infections. Therefore, it is unclear if the primary endpoints were traditional CV risk factors as stated on page 6, or the parameters of oxidative stress and inflammation studied in the already published study.

Response: Sample size calculation is detailed in page 5

1.2. Comment: Page 5, second paragraph: “During the study period patients were instructed not to drink any other fresh fruit juice at home”. In this case, the patients assigned to the placebo group were at a disadvantage because they can not benefit from anti-inflammatory and antioxidant effects of these fruit juices. Therefore, is not surprising that in the placebo group, pulse pressure and plasma levels of triglycerides significantly increased (p=0.02 and p=0.04, respectively) and systolic blood pressure also changed to worse, although this increase was not significant (see table 1 and 2). I wonder if any of the statistically significant differences between groups might be explained for both factors: an improvement in the PJ group and an impairment in the placebo group. The authors should discuss this point this in the text.
Response: We add a reference in page number 9.

1.3. **Comment**: "Page 6, “statistics”: " The t-test for independent samples, or Mann Whitney test when appropriate, were used to detect differences in continuous variables between treatment groups”. When was appropriate?. Was normality of distribution analyzed?. If yes, what test was used?

**Response**: In Cases we had less than 30 observations and no normally distribution we used the Mann – Whitney U test, otherwise we used the T-test.

1.4. **Comment**: Page 7: “Characteristics of the 101 patients are described in our previous paper [18]”. In my opinion, these characteristics must be expressed in detail, using a table, in the present manuscript

**Response**: The characteristics of the study population was detailed in the text (page 7). Expresses the date with a table (which appear in our previous article) in addition to the text will create a duplicity which seems to be unnecessary.

1.5. **Comment**: At baseline, the number of subjects with SBP> 140/90 mm Hg, those with HDL values < 40 mg/dl and those with triglycerides > 200 mg/dl were always higher in the PJ group (respectively: 26 vs. 5 / 54 vs. 23 / 20 vs. 10). Could this translate a defect in the randomization? In any case, this would facilitate positive outcomes in the group of PJ, since more ill patients, are also, patients which more can improve. The authors should discuss this point in the text.

**Response**: The analysis of patients with pathological levels as detailed above included only patients for whom no changes were done, during the study period, in the number of medication therapy (for hypertension when examined SBP and for lipid profile when examined HDL and TG). A stable medication therapy during the study, which was more pronounced in the PG group compared to the placebo, probably due to the beneficial effect of the PG, is responsible to the higher number of patients in the PG group compared to the placebo that were suitable for this analysis. Furthermore, at baseline, we did not demonstrated any difference between the two groups regarding demographic and clinical parameters including no difference in blood pressure nor in lipid profile, which indicated the successful of our randomization.
Author's response to reviews
Page 4 of 5

1.6. Comment: Was there any patient with DBP > 90 mm Hg at baseline? If yes, it must be included in table 1, in the same way that patients with SBP > 140 mm Hg were included.

Response: We had only 2 patients in the PJ group and 3 patients in the placebo group with DBP > 90 mm hg, as such we did not included them in table 1.

1.7. Comment: Table 1: P value for the comparison PJ vs. placebo at baseline must be included, in the same way that at 12 m. It seems that patients in the group of PJ are much more hypertensives than those of the placebo group. If it is the case, it may be included in the table and discussed in the text.

Response: Done

1.8. Comment: "Please change mm/Hg by mm Hg all through the text and in table 1."

Response: Done

Reviewer 3:

1. Major Compulsory Revisions:

1.1. Comment: “The authors emphasize the apparent beneficial effect of PJ on several biomarkers, but in reality there was only limited statistical difference between the two treatments. It is true that the PJ-treated group exhibited improved SBP, PP, HDL and TG values when compared to baseline. However, only HDL (and barely TG) showed real differences in a between-treatment comparison, something that was not considered in the Discussion. The section must be modified in order to cover these issues.”

Response: we added a remark in the discussion section, page 9, first paragraph.

1.2. Comment: "No mention is made to the estimated discriminating power for variables other than SBP (page 5, third paragraph). How can the authors be sure that the lack of effect on the different outcomes was not influenced by insufficient statistical power?"

Response: we calculated the sample size according to the parameter with the lower expected difference before and after 12 months of PJ consumption. As such, we can be sure that the sample size is sufficient also to the other parameters.
Author's response to reviews

Page 5 of 5

1.3. Comment: "The composition of the placebo juice must be reported in the Methods section."
   Response: Done, page 6, first paragraph

2. Minor Essential Revisions

2.1. Comment: "Please provide formal details regarding the approval by the ethics committee"
   Response: Done, page 5, first paragraph

2.2. Comment: "Page 5, fourth paragraph: the sentence “Patients who did not drink the juice at least 3 times per week were excluded” is confusing. Page 5, 2nd paragraph, states that 3 times/week was the programmed dose (page 5, 2nd paragraph). Is that correct? Did you allow greater doses?"
   Response: Should be "… at least 3 times were excluded" – corrected.

2.3. Page 8, last paragraph: the “anti-atherogenicity properties” of PJ have not been mentioned before in the text. Please include this information in the Background chapter.
   Response: Done, end of page 3

3. Discretionary Revisions

3.1. Comment: "Page 8, paragraphs 1 and 2: Shouldn’t it read “Table 1” instead of “Table 2”?"
   Response: Corrected

3.2. Comment: "Please correct typos in references 11 and 12 (page 13)."
   Response: Done