Original Article

The effect of thyme honey on anemia in hemodialysis patients

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ARTICLE INFO

Received 11 October 2019
Accepted 16 February 2020
Published 01 April 2020
Available online at:
http://npt.tums.ac.ir

Key words:
anemia;
hemodialysis;
thyme honey

ABSTRACT

Background & Aim: Anemia is one of the most common complications of chronic renal failure, which is associated with increased fatigue, dyspnea, depression, malaise, prolonged hospitalization and mortality. Some patients are resistant to erythropoietin therapy. Therefore, the purpose of this study was to determine the effect of thyme honey on anemia in hemodialysis patients.

Methods & Materials: The present clinical trial was conducted in 2018 at Kowsar Hospital of Semnan on hemodialysis patients randomly selected with convenience sampling method within two groups of case (n=28) and control (n=27). The case group received thyme honey (33 g) daily for one month. The control group did not consume thyme honey. Blood samples were taken from the patients before and after the intervention with 10-hour fasting and then blood parameters were assessed.

Results: The mean ±SD reduction rate in hemoglobin level was 0.46±0.96 in the case group and 0.23±3.15 g/dl in the control group, but no significant difference using independent t-test (P=0.758). The mean±SD reduction rate in hematocrit percentages was 1.20±2.78 in the case group and 0.11±8.53 in the control group, but there is no significant difference between the two groups using independent t-test (P=0.590).

Conclusion: Results showed that daily administration of 33 grams of thyme honey had no effect on anemia in hemodialysis patients. Further studies are recommended with modification of the methodology regarding the type, dose, duration and instructions of honey consumption for better results.

Introduction

Chronic renal failure (CRF) is a global health problem whose prevalence rate is on the rise (1). The CRF refers to kidney damage or an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m² lasting for three months or more (2). End-stage renal disease (ESRD) is the last stage of CRF, a loss of about 90% of active kidney function. In other words, the term ESRD is used when the patient must use alternative kidney therapies, such as transplantation or dialysis, to preserve life (3). According to a report by Fresenius Medical Care in 2018, the approximate number of dialysis patients in the world was 3362000, of which 88% (n=2944000) underwent hemodialysis (4). In Iran, there have been approximately 58000 ESRD patients up to March 2017, of whom 29200 patients underwent hemodialysis (5).

In the patients with CRF, the anemia is very common and the severity of anemia increases with the progression of renal failure. Evidence suggests a direct linear relationship between the rate of decrease in GFR and the rate of decrease in hemoglobin level (6). It has been estimated that 90% of patients with the GFR of less than 25-30 ml/min will experience the anemia (7).

The World Health Organization (WHO) defines anemia as hemoglobin level less than 13 g/dl in men and less than 12 g/dl in women (6). In the hemodialysis patients, the hemoglobin levels over 13 g/dl are not associated with a decrease in cardiovascular

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DOI: https://doi.org/10.18502/npt.v7i2.2738

*Please cite this article as: Mousavi Kiasari S.A, Nobahar M, Ghorbani R, Tamaddon M.R. The effect of thyme honey on anemia in hemodialysis patients. Nursing Practice Today. 2020; 7(2):151-160
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disease and their mortality rates, and their recommended hemoglobin levels are over 11 g/dl (8).

The anemia develops in the patients with CRF by a number of factors, including decreased erythropoietin production, impaired iron homeostasis, elevated plasma hepcidin hormone (which inhibits dietary iron absorption), plasma uremic environment, decreased erythrocyte life, and other metabolic factors. The anemia can be exacerbated in the hemodialysis patients due to factors such as dietary restriction, vitamin deficiency, and failure to absorb dietary iron, oxidative stress, chronic bleeding from platelet dysfunction, entrapped and wasted blood during hemodialysis technique and repeated blood sampling (9).

The progression of CRF and exacerbation of anemia increase the risk of some dangerous cardiac complications, including angina, left ventricular hypertrophy, and heart failure in these patients. These cardiac complications as a cycle (cardiorenal anemia syndrome, CRAS) can increase renal failure and thereby exacerbate anemia (10).

Hematopoietic stimulants, such as erythropoietin, are used currently to improve anemia associated with renal failure. Evidence suggests that elevated hemoglobin levels close to normal in these patients are associated with a decrease in the risk of cardiovascular disease (CVD) and an increased survival rate. However, clinical trials have shown that high levels of erythropoietin may increase the risk of CVD and mortality in these patients (11). In addition, the therapeutic effects of erythropoietin are often limited due to iron deficiency in the hemodialysis patients (12). Given the high impact of oxidative stress on accelerating blood cell destruction and exacerbating anemia, especially in chronic conditions, new therapeutic approaches have been directed toward the use of antioxidants as potential agents in the improvement of anemia (13).

The presence of a variety of flavonoids, phenols, vitamins, minerals and antioxidant enzymes and other factors in the honey composition made this product have remarkable anti-inflammatory and anti-oxidant properties. Compared to other famous antioxidants, such as vitamins E and C, honey more effectively and efficiently neutralizes free radicals. Given these characteristics, the use of honey as an antioxidant may have more beneficial results than the vitamin E or C evaluated in various studies for their therapeutic effects that showed unsatisfactory results (14). Raw honey is considered as a suitable complementary and alternative medicine in controlling diseases, reducing infection and promoting human health due to the presence of many nutrients and bioactive compounds that lonely or synergistically has significant therapeutic effects (15).

Previous animal studies reported that daily administration of raw honey could improve hematological parameters and increase hemoglobin levels and red blood cell counts (17, 16). In addition, honey administration in rats with lead-induced anemia prevented the development of lead-induced anemia, possibly due to elevated serum iron levels and bone marrow stimulation (17). Contrary to previous studies, Aliyu et al. (2012) in Pakistan reported that the administration of acacia honey significantly reduced blood hemoglobin levels in rats (18).

In human samples, it is showed that oral administration of honey for two weeks in healthy individuals significantly increased serum levels of iron, copper and vitamin C and significantly reduced plasma ferritin levels and increased insignificantly the plasma hemoglobin level (19). According to Chauvin, Bogdanov stated that the honey administration with warm milk in infants aged four to eight days increased blood hemoglobin level after a week (20).

Abdul Rahman et al. (2016) studied children with acute lymphoblastic leukemia (ALL) and found that the oral administration of 2.5 g/kg of clover honey twice a week for three months increased the level of hemoglobin without causing side effects. In this cross-sectional study, discontinuation of taking honey at a later period decreased
hemoglobin level, total neutrophil count, and platelet count (21).

The chemical composition and color of honey varied widely depending on the plant origin and geographical area, the method of preparation and maintenance, and other factors. Darker honey contains more levels of minerals (especially iron), phenol and flavonoids than lighter honey. As a result, these honeys have higher antioxidant content and are able to scavenge more free radicals. The presence of different chemical compositions in various honeys has caused differences in the therapeutic properties of these products (22). For example, it is showed that only dark honey returned hemoglobin levels to normal in rats with induced anemia, and light honey was unable to improve the anemia (20).

Thyme honey with a pleasant aroma and taste is one of the most popular and widely used monofloral honeys globally (23). Studies on the thyme honey suggest that this honey is as significant as other dark honeys containing phenol, flavonoids and vitamin C, and has higher antioxidant properties than other honeys. In addition, the thyme honey has been found to be somewhat inhibitory activity for angiotensin-I-converting enzyme (24). However, the administration of honey with a higher fructose/glucose ratio less causes a rapid rise in blood glucose. This property is more evident in monofloral honey. Among the several honeys studied, the thyme honey, which has a different fructose/glucose ratio, has induced the lowest post-administration glycemic response, which is important in patients with diabetes (25).

Although previous studies have shown the therapeutic and prophylactic effects of honey associated with anemia in non-dialysis patients, further studies are needed to generalize these results to hemodialysis patients, who have special conditions due to hemodialysis procedures. Considering the therapeutic importance of the various honeys that have been underestimated in previous studies, and due to the dark color and chemical composition of thyme honey that contains significant levels of minerals such as iron and copper, significant level of vitamin C, high antioxidant level and higher fructose/glucose ratio (important in hemodialysis patients with diabetes), pleasant taste and odor, abundance, and availability of this type of honey in Iran, the thyme honey was used to conduct the present study. Therefore, this study was performed to determine the effect of thyme honey on anemia in hemodialysis patients.

Methods

The present clinical trial was conducted in 2018 at Kowsar Hospital of Semnan on 55 hemodialysis patients randomly selected with convenience sampling method, who participated in the study voluntarily and with informed consent. For sampling, the nurses of the relevant ward performed face-to-face interview and then encoded demographic information questionnaires were completed. In fact, each questionnaire represented a case or control sample. Then, the researcher, who was unaware of the characteristics of the subjects in the questionnaire, randomly divided the questionnaires into two case and control groups, as even or odd, and the samples were randomly divided into two groups of case (n=28) and control (n=27). The case group received thyme honey (33 g) daily for one month, and the control group did not consume thyme honey. Blood samples were taken from the patients before and after the intervention with 10-hour fasting and then blood parameters were assessed. Subsequently, investigations were performed on 42 specimens following the withdrawal of 13 patients from the study (Figure 1).

Inclusion criteria were willingness to participate in the study, sign informed consent, history of hemodialysis treatment three times a week for three hours or more, history of hemodialysis for at least three months, and absence of allergy to honey administration. A history of medication prescribing, if prescribed for at least three months, was not an obstacle to study (26).

Patients with mental disorders, cancer, liver failure, hepatitis were not included in
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the study. Exclusion criteria were severe emotional stress, irregular administration of thyme honey, change in prescription drugs, any hospitalization, fasting intolerance, transfusion of blood or blood products, migration, history of kidney transplant, and death of patients.

The first step in the research was to produce fresh and raw thyme honey, the honey that the bee had to feed mainly from the thyme and with minimal processing and harvesting. Therefore, the honey samples were sent to the Razi Food Lab (Urmia) before purchasing honey to determine the purity of honey. The results of tests on parameters such as acidity, moisture content and the ratio of sugars present showed that the aforementioned carnelian thyme honey had been collected from the Taleghan Plains, and had the required standards and sufficient purity (Table 1).

The prepared thyme honey samples were packed in 1-kg containers. Honey dishes were labeled to control and regulate the amount of honey administered, so that everyone knew how much dose of honey they should consume by the end of ten days. Daily consumption dose was 33 g of honey as fasting. The honey was given to people on dialysis in the morning immediately after dialysis. Special spoons were made, each filled spoon containing 30 to 35 g of thyme honey.

The statistical population was hemodialysis patients. The research units were hemodialysis patients in the hemodialysis ward at Kowsar Hospital of Semnan. The hemodialysis patients were interviewed after obtaining permission from the University Ethics Committee and obtaining permission from hospital authorities and the hemodialysis ward at Kowsar Hospital of Semnan. Patients who met the inclusion criteria were identified. After providing necessary explanations of the importance of the research and methodology, the patients were enrolled in the study and signed informed written consent. The patients in the case group were visited and trained practically and individually on how to use and dose of honey administration and other issues regarding lifestyle and dietary changes; then the emergency telephone numbers were exchanged reciprocally for greater accountability and coordination. The control group also received the necessary hints about keeping constant their eating habits and lifestyle.

Baseline sampling was performed with a minimum of 10 hours of fasting for all participants in the study. The researcher and one of the ward's nurses took arterial blood samples before heparin injection. The day before sampling, the necessary information about fasting was obtained by telephone. The sampling would not be performed if there were any doubt as to the non-observance of the fasting time. The first (before the intervention) and the second (one month after the intervention) sampling time points were at a specific and similar time. Sampling from all patients took one week at each stage.

The case group received a labeled container containing 1 kg of thyme honey with a special spoon, and began to consume honey one day after the first sampling. During this one month, they were repeatedly contacted, either by telephone or in person, to control their consumption dose and to emphasize the administration of honey. In addition, the patients brought their honey containers with them on the tenth and twentieth day and the last day to evaluate the amount of honey administered and to explain the necessary points. After one month, the honey containers were collected and blood samples were taken again in accordance with the conditions of the first sample, and sent to the Khatam al-Anbia Laboratory in Semnan to evaluate the relevant parameters. Hemoglobin and hematocrit levels were measured using KX21 cell counter, Germany. Concerning the accuracy and precision of the results, after calibrating the cell counter and sending the R&D control sample of the Biotechne brand with the expected mean of 12±0.5 and 33±2.4 respectively for hemoglobin level and hematocrit percentage, the results of the samples were 12 g/dl for hemoglobin level,
and 35.2 for hematocrit percentage, indicating the accuracy of device calibration and measurement accuracy.

Ethical considerations in this study included obtaining clinical trial approval under Project No. 1206, endorsing the Ethics Committee of Semnan University of Medical Sciences (IR. Code.SEMUMS.REC.2018.4) on July 24, 2017, registering on the Iranian Registry of Clinical Trials (IRCT201705266318N9), obtaining approval from Nursing Care Research Center and Research Council of Nursing, obtaining a letter of reference to hospital officials, obtaining a chief and doctor's approval, expressing the research objectives, obtaining patients' informed written consent, ensuring the confidentiality of information collected. The patients participated voluntarily in the study, and could leave the study freely at any time, with no change in the behavior of the medical staff or the treatment and care regimen. Ethically, the hemoglobin level and the hematocrit percentage were measured in a private laboratory out of hospital as free of charge for all patients on both sampling time points. The results of the tests were informed to all patients, who were satisfied, by the researcher. At the end of the study, the thyme honey was also given to the control group.

Data were analyzed using SPSS 23 software. Chi-square test was performed to investigate the homogeneity of the two groups in terms of nominal qualitative variables. Normality of data was evaluated using Shapiro-Wilk test. Independent t-test was used to test the quantitative trait in two independent groups with normal distribution, and Mann-Whitney test was applied for data with non-normal distribution in at least one of the groups. Significance level was considered to be 0.05.

Figure 1. Consort flow diagram of the study
Results

The mean (± SD) age was 58.9±13.1 in the case group and 64.1±11.3 in the control group, but this difference was not significant (P=0.170). In addition, 59.1% of the case group and 50.0% of the control group were male. Sex distribution was homogeneous in the two groups (P=0.555). The mean (±SD) BMI was 25.2±6.1 kg/m2 in the case group and 24.9±5.3 kg/m2 in the control group. The body mass index distribution was not significantly different between the two groups (P=0.960) (Table 2).

The mean (±SD) reduction rate in hemoglobin level was 0.46±0.96 in the case group and 0.23±3.15 g/dl in the control group, but not significant difference using independent t-test (P=0.758). The mean (±SD) reduction rate in hematocrit percentages was 1.20±2.78 in the case group and 0.11±8.53 in the control group, but there was no significant difference between two groups using independent t-test (P=0.590) (Table 3, Figures 2 and 3).

The paired t-test showed a significant decrease in the mean hemoglobin level in the case group (P=0.036) but no significant decrease in the control group (P=0.747). In addition, the paired t-test revealed no significant decrease in the mean hematocrit percentage in the case group (P=0.056) and the control group (P=0.955) (Table 3).

Table 1. Laboratory characteristics of honey used in research

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Specifications of thyme honey</th>
<th>Standard rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of regenerative sugars</td>
<td>76.75%</td>
<td>Minimum 65%</td>
</tr>
<tr>
<td>Fructose to glucose ratio</td>
<td>1.1%</td>
<td>Minimum 0.9%</td>
</tr>
<tr>
<td>Sucrose percentage</td>
<td>1.3%</td>
<td>Maximum 5%</td>
</tr>
<tr>
<td>Diastases</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Acidity</td>
<td>16.9 mg/ kg</td>
<td>Maximum of 40 mg/ kg</td>
</tr>
<tr>
<td>Humidity</td>
<td>14.1%</td>
<td>Maximum 20%</td>
</tr>
<tr>
<td>Hydroxy Methyl furfural (HMF)</td>
<td>4.9 mg/ kg</td>
<td>Maximum 40 mg/ kg</td>
</tr>
</tbody>
</table>

Table 2. Distribution of demographic characteristics of hemodialysis patients in two groups

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Case (N=28)</th>
<th>Control (N=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;50</td>
<td>8</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>4</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>60-69</td>
<td>6</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>&gt;70</td>
<td>4</td>
<td>18.2</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>13</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>40.9</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>&lt;25</td>
<td>15</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>25-29.9</td>
<td>2</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>&gt;30</td>
<td>5</td>
<td>22.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Yes</td>
<td>8</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>14</td>
<td>63.6</td>
</tr>
</tbody>
</table>

*: Independent T  **: Chi Square  ***: Man Whitney

Table 3. Mean and standard deviation of hemoglobin and hematocrit in hemodialysis patients before and after the intervention and their changes in the two study groups

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Group</th>
<th>Before intervention</th>
<th>After intervention</th>
<th>Before and after changes</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Case</td>
<td>11.8</td>
<td>1.4</td>
<td>11.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.9</td>
<td>2.1</td>
<td>11.7</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>P value*</td>
<td>0.811</td>
<td>0.418</td>
<td>0.758</td>
<td>-</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>Case</td>
<td>36.5</td>
<td>3.7</td>
<td>35.3</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>37.7</td>
<td>6.3</td>
<td>37.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>P value*</td>
<td>0.468</td>
<td>0.074</td>
<td>0.590</td>
<td>-</td>
</tr>
</tbody>
</table>

*: Independent t-test  **: Paired t-test
Discussion

The results of this study showed that daily administration of 33 g of thyme honey for one month in hemodialysis patients had no effect on hemoglobin level and hematocrit percentage. The reason for these findings is probably due to the specific conditions of the research units (the hemodialysis patients), but not due to the type of intervention (thyme honey), resulting in no effect of the intervention. Most previous studies that reported the positive effects of honey administration on slightly or significantly elevated hemoglobin levels mainly used amber and dark honeys, such as Manuka, Tualang, Clover and Carob honey (16, 17, 21). These honeys have high levels of phenolic compounds, minerals (iron, copper, etc) and antioxidant properties, which have hepatoprotective effects and positive effects on red blood cell production by stimulating bone marrow (17). The reason for the significant decrease in hemoglobin level in the study by Aliyu et al. (2012) in Pakistan can be attributed to the type of honey used, the acacia honey with watery white color (18). This study used...
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Thyme honey, which their physical and chemical properties are consistent with dark honey (27). The major difference of this study from previous studies is that all previous studies were carried out on non-hemodialysis samples. In addition, issues such as the loss of nutrients, minerals and vitamins absorbed into the blood by the administration of honey due to hemodialysis technique were not raised in previous studies.

In the hemodialysis technique, many valuable and useful nutrients in the blood and many vitamins, especially water-soluble ones such as vitamin C, small molecules, and proteins that are unable to bind to proteins, are lost when performing exchanges between soluble substances in the patient's blood and hemodialysis fluid through the semipermeable membrane of the filters (28). When using high-flux membranes, depending on the type of filter, molecules with molecular weights between 10 and 50 kDa are also capable of passing through the membrane pores of these types of filters (29), so most compounds present in thyme honey that are capable of dissolving in water and having much lower molecular weight are easily wasted during hemodialysis.

In previous studies, the consumption dose of honey was between 0.5 and 2.5 g/kg of body weight. In some studies, high doses increased the level of glycosylated hemoglobin (30). Considering the inclusion of some people with diabetes in this study, and generally considering the least possible risk for hemodialysis patients who are physically and mentally sensitive, the minimum consumption dose was considered for the thyme honey (0.5 g/kg). Given the above, and considering the probability of wasting a large percentage of thyme honey nutrients after each hemodialysis session, the dose of honey seems to have been very low, and at least several times the dosage taken should be considered in order to show the beneficial and effective effects of honey. In addition, the minimum duration of honey administration to improve health in the general population has been between two and three weeks in various studies (20). In the present study, with hemodialysis performed every other day in the research units, although the patients were prescribed thyme honey for one month, the actual time to effect and make changes was much less than one month. It seems that the duration of honey administration should be prolonged in order to obtain better results in these patients with substantial changes in the research method.

Another notable point in the discussion of results is the facts that research units (non-hemodialysis patients) in previous studies have prescribed honey in syrup, which is both easier and faster to administer and absorb. In the present study, due to the limitation of hemodialysis patients in fluid administration and prevention of overweight between the two hemodialysis sessions, the research units administered raw thyme honey. Despite the pleasant taste and smell of thyme honey, a full spoon of raw thyme honey in one serving is difficult due to high sweetness and the research units may prescribe honey less than the recommended dose. Future studies need to design the study method so that research units consume honey as a syrup, for easier consumption and faster absorption.

Although the hemodialysis patients are highly susceptible to anemia due to plasma uremic environment, oxidative stress, chronic systemic inflammation, and bioincompatibility between blood and membranes and hemodialysis fluid, the results of this study may have been influenced by factors such as repeated blood sampling, blood loss while detaching the patient from the hemodialysis device, or latent bleeding and other things, which were ignored by the researchers.

It was impossible to fully control patients' diet, fasting status, precise dose of honey administration, blood loss during connection or withdrawal from the hemodialysis device, any patients' physical and mental stress, use of a type of hemodialysis device or a type of hemodialysis membrane for all patients during the study period, which were of the limitations of the present study.
Since the hemodialysis is one of the major sources of oxidative stress in these patients, with a 14-fold increase in free radical levels occurring in these patients after hemodialysis (31), and given its effect on exacerbating anemia, if future studies are designed to allow interventions during hemodialysis, better outcomes may be obtained by neutralizing free radicals produced during hemodialysis by removing the restrictions associated with water and honey administration. Therefore, better results may be obtained by administering higher antioxidant content (monofloral honey) dissolved in a solvent such as milk, with multiple doses (100 g of honey per one liter of milk) as milk/honey syrup repeatedly during hemodialysis.

Acknowledgment

This study has been adapted from an MD thesis in Critical Care Nursing with a registration number on the Iranian Registry of Clinical Trials IRCT201501166318N9 and an approval of the University Research Council (Project Number 1206). The authors would like to thank the Nursing Care Research Center and the Deputy of Research and Technology at Semnan University of Medical Sciences for supporting the implementation and costs of the project, as well as the Hospital officials and Hemodialysis Ward at Kowsar Hospital of Semnan for assistance with the study and all colleagues and patients in the Hemodialysis Ward.

Conflict of Interest

The Authors declare no conflicts of interest with respect to the research, authorship, and publication of this article.

References

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