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Unfermented grape juice reduce genomic damage on patients undergoing hemodialysis

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ABSTRACT

Chronic kidney disease (CKD) patients in dialysis (HD) are considered to be submitted to a continuous oxidative stress. This stress can cause damage on DNA and, consequently, contribute to the high levels of DNA damage observed in these patients. Due to the well-known role of polyphenols as antioxidant agents we proposed its use to reduce the levels of genotoxicity present in HD-CKD patients. The objective of this study was to evaluate the antigenotoxic effects of unfermented grape juice (UGJ) on HD-CKD patients. The levels of DNA damage were analyzed using different biomarkers, such as breaks and oxidized DNA bases by the comet assay, chromosome damage by the micronucleus test. In addition, TEAC (Trolox equivalent antioxidant capacity) was also evaluated. Thirty-nine patients were followed for six months, of whom 25 were supplemented by UGJ and 14 were not supplemented. The obtained results showed a significant decrease in the underlying levels of oxidative DNA damage, in the supplemented group. Regarding the clinical parameters, LDL and cholesterol, were significantly reduced in the patients studied after the supplementation period, although cholesterol was also decreased in the non-supplemented patients. In conclusion, in our studied group the supplementation with UGJ reduced the levels of oxidative DNA damage of HD-CKD patients.

1. Introduction

Oxidative stress is the result of an accumulation of reactive oxygen species (ROS), together with a low antioxidant capacity, that leads to biochemical alterations causing structural and functional modifications of these biomolecules (Massy et al., 2009). Many studies have focused on the detection of oxidative stress in patients with renal alterations to determine whether this is an underlying cause/effect mechanism in chronic kidney disease (CKD). At this point, it must be indicated that CKD patients under dialysis (HD) are submitted to a continuous oxidative stress (McDonald et al., 2014). The HD process contribute to the elimination of plasma antioxidants and together with the dietary restrictions of these patients, the reduced antioxidant enzyme activity, and iron and erythropoietin supplements, these patients became a group with potentially high levels of ROS (Spormann et al., 2008). These reasons support the view that HD process induces oxidative stress in CKD patients and it has been reported that HD-CKD patients show significantly higher levels of oxidative DNA damage than CKD predialysis (PD) patients (Corredor et al., 2015). One of the hypotheses explaining this fact is that, during the dialysis session, the contact blood-membrane induced the activation of macrophages, losing antioxidant capacity and contributing to enhanced oxidative stress in CKD patients. In this scenario the use of antioxidants supplements can be helpful to this type of patients.

It is well known that phenolic compounds act as antioxidant, specially due to their ability to donate hydrogen or electrons and prevent the oxidation of various compounds, particularly fatty

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acids and oils (Gülçin, 2012). Fruits, especially those with red or blue color (such as grapes, plums and cherries), are the most important sources of polyphenols (Stratil et al., 2007). In grapes, flavonoids are primarily located in the epidermal layer of berry skin and in the seeds (Waterhouse, 2002). Flavonoids are the main group of soluble phenolic compounds in grapes, as well as the main contributors of the biological activities in products derived from grapes (Conde et al., 2007). It has been reported that ingestion of unfermented grape juice (UGJ) as a polyphenol-rich dietary supplement exerts hypolipidemic, antioxidant, and anti-inflammatory effects in hemodialysis patients (Castilla et al., 2006). In fact, increased uptake of food-based antioxidants can be a promising alternative measure to reduce oxidative cell damage and stress response (Weisel et al., 2006). In HD patients, few studies (Castilla et al., 2006, 2008; Spormann et al., 2008; Alipour et al., 2012; Janiques et al., 2014) have evaluated the effects of polyphenolic-rich fruit juices on antioxidant capacity, and oxidative stress. No previous studies have been carried out to determine its effect on the levels of genetic damage in such patients. Therefore, the aim of this study was to evaluate the effect of UGJ on the levels of genomic damage in CKD patients under HD by analyzing markers such as genomic/oxidative DNA damage (comet assay) and chromosome damage (micronucleus test). Additionally, variations on TEAC (Trolox equivalent antioxidant capacity) values were also determined.

2. Materials and methods

2.1. Study population

The study involved a total of 39 CKD patients undergoing hemodialysis three times per week, with 3:30—4 h per session. Patients were recruited at the hospital Fundació Puigvert (Barcelona, Spain). Two blood samples were obtained for each patient before the HD session, before and after 6 months. Patients were randomly distributed in 2 groups: UGJ and reference. During that period of time, 25 CKD patients were supplemented, during the last half hour of each dialysis session, with 100 mL of unfermented grape juice (UGJ), assuming that they did not changed their food intake habits during the studied period. A descriptive of the general characteristics of the studied population is indicated on Table 1. Medications and supplements administered to these patients during the follow up are indicated in Table 2. Clinical data was recovered directly from medical history, and clinical parameters (Table 3).

Standard blood analysis included the determination of calcium, phosphorus, glucose, cholesterol, triglycerides, albumin and hemoglobin, among other parameters. Moreover ferritin, iron, transferrin saturation index, parathyroid hormone and C-reactive protein were also analyzed. The erythropoiesis stimulating agents (ESA) resistance index (ERI) was determined as the weekly weight-adjusted ESA dose (IU/week/kg) divided by the product of the patient's weight (Kg) and the hemoglobin level (g/dL). A conversion ratio of 1:200 was used to convert the darbepoetin dose (mcg) to international units (IU) of erythropoietin. All individuals participating in the study provided written informed consent, and blood samples were collected under protocols approved by the Ethics Committee of the Puigvert Foundation. Blood samples were sent to the Universitat Autònoma of Barcelona and immediately processed to analyze the levels of genomic damage and the antioxidant capacity.

2.2. Unfermented grape juice

The UGJ concentrate administered to the patients, was purchased from Concentrados Pallejà S.L. (Riudoms-Tarragona, Spain). The UGJ was unpasteurized, fresh concentrate to avoid losing the antioxidant properties. The polyphenol composition was assessed at the Instituto de Investigación en Ciencias de la Alimentación (CSIC, Madrid). To determine the composition of the UGJ, two different methods were applied (Monagas et al., 2006): (1) the method of total polyphenols, based on oxidation in basic medium of phenol hydroxyl groups fear the Folin-Ciocalteu reagent; and (2) the method of total anthocyanins, based on a colorimetric pH change. The results are expressed in mg of gallic acid/L. From these studies it was concluded that the UGJ contained: total polyphenols 5888 ± 262 mg/L and total anthocyanins 1515 ± 98 mg/L. The levels of potassium were 7.5 mEq/100 mL and correspond to the 6.6% of the estimated daily intake of potassium in hemodialysis patients. The UGJ concentrate was bottled in 1 L-cans, stored in a refrigerator at 4 °C, and administered by the nurses before the end of each hemodialysis session.

2.3. Comet assay

DNA breaks present in peripheral blood lymphocytes were measured using the comet assay performed following the standard protocol, as previously described (Singh et al., 1988; Stoyanova et al., 2010) with minor modifications. Briefly, isolated lymphocytes from 2 mL of blood from each patient were cryopreserved until use, in 500 μL of medium containing 90% serum and 10% DMSO. Comet assay was carried using Gelbond® films (GF) instead of microscopic slides as a support for the agarose gel. The use of hydrophilic films facilitates the rapid processing of numerous samples, increasing the efficiency of the alkaline comet technique, without sacrificing the reliability or sensitivity of the assay (McNamee et al., 2000; Azqueta et al., 2013). Lymphocytes were isolated using Ficoll—Paque density gradient from 500 μL of whole blood; cells were adjusted to a concentration of 17,800 cells in 25 μL

Table 1
General description of the studied groups.

	Supplemented patients (N = 25)	Reference patients (N = 14)
Gender (men/women) (%)	15 (60)/10 (40)	9 (64.3)/5 (35.7)
Age (years) (mean ± SE)	66.16 ± 2.55	59.71 ± 4.61
BMI (mean ± SE)	25.47 ± 1.22	23.93 ± 1.06
Time in HD (months) (mean ± SE)[a]	36.24 ± 5.50	16.57 ± 7.36*
RT previous (% yes/no)	8/92	21.4/78.6
Hypertension (%yes/no)	92/8	92.9/7.1
CV pathology (%yes/no)	56/44	71.4/28.6
Previous cancer (%yes/no)	44/56	35.7/64.3
Diabetes mellitus (%yes/no)	32/68	35.7/64.3
Dyslipidemia (% yes/no)	76/24	64.3/35.7

SE, standard error;; *t*-test, $^*P < 0.05$.
[a] HD time before treatment.