Aspartame and Platelets in Type II Diabetic Patients

Arbind Kumar Choudhary

ABSTRACT

Background: For type II diabetes (T2D) subjects to better regulate carbohydrate consumption and manage blood glucose levels, a non-nutritive sweetener (aspartame) is prescribed as an alternative to natural sugar. Previous studies show that there was a 68% rise in the probability of aspartame consumers developing T2D compared with non-consumers. In diabetes and inflammation, deformed red blood cells (RBCs) and atypical fibrin fibre formation or an altered fibrin structure are especially prevalent.

Objective: The aim of this study was to investigate, in subjects with T2D taking aspartame, clot viscoelasticity and platelet structure.

Methodology: Blood was drawn from 12 T2D subjects from the diabetic clinic at the Steve Biko Academic Hospital, South Africa. Blood was used to perform a full blood count, thromboelastography (TEG) and scanning electron microscopy (SEM).

Results: SEM showed increased platelet activation and abnormal TEG parameters in T2D consuming aspartame.

Conclusion: A hypercoagulable state can increase the risk of thromboembolic complications and an increased incidence of vascular disease. This knowledge may be used to build awareness among consumers of aspartame.

Keywords: Aspartame, blood, coagulation, diabetes, platelets.

I. INTRODUCTION

Aspartame consists of two amino acids; L-phenylalanine and L-aspartic acid are esterified into methyl alcohol and generate about phenylalanine (50%), aspartic acid (40%), and methyl alcohol (10%) by weight after being metabolized in the body [1]. The levels of its metabolites are increased in the blood following aspartame's intake [2]. The body uses these components in the same way as natural foods such as milk, fruit, and vegetables [3]. For type II diabetes (T2D) subjects to increase carbohydrate intake regulation and control blood glucose levels, aspartame is prescribed as an alternative to natural sugar [4]-[6]. Aspartame is used in a broad range of food and beverage items, such as table sweeteners, desserts, milk, ice cream, baked goods, jelly, preserves, marmalade, soft drinks, candy, mustard sauces, and medication [3], [7]. Acceptable daily intake (ADI) for aspartame 40 mg/kg body weight was developed by the European Food Safety Authority (EFSA) Panel [8]. Using non-nutritive sweeteners (aspartame) in T2D subjects is advantageous for eating sweet foods and drinks without raising calories. On the other hand, the risk of developing T2D was 68% higher for aspartame consumers than non-consumers, based on their food frequency questionnaires [9]. There are important ties between oxidative stress, altered inflammatory markers (inflammation), and T2D development [10]. We also understand that deformed red blood cells (RBCs) and the formation of atypical fibrin fiber or an altered fibrin structure are especially prevalent in Diabetes and inflammation [11]-[14].

In view of this, in the present work, we hypothesized that aspartame usage may cause additional stress and damage the blood clotting mechanism in subjects with type II diabetes consuming aspartame as shown in Fig. 1. Hence, we studied clot viscoelasticity and platelet structure in patients with T2D consuming aspartame.
II. METHODOLOGY

A. Ethical Declaration

This was a prospective observational study and was approved (Ethics Reference no-68/2016 Dated-31/03/2016) by the Research Advisory Ethical Committee, Faculty of Health Science Research, University of Pretoria, South Africa.) The study was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before the blood samples were drawn.

B. Study Design

T2D subjects consuming aspartame were recruited to participate in this study. Twelve T2D subjects (males and females aged 45 to 75 years) consuming aspartame Table I were randomly selected from the Steve Biko Academic Hospital Diabetic Clinic. Concomitant medication prescribed to T2D subjects in this study has no potential effect on platelet function and fibrin clot, as investigated earlier [10]. T2D subjects who were re-diagnosed with anaemia and phenylketonuria were removed from this study. A history of immunocompromised status, as well as any herbal supplements or corticosteroids, anti-inflammatory, anti-platelet, or blood-thinning drugs taken two weeks before the study was excluded. Blood samples of citrate and EDTA were obtained from volunteers who met the inclusion requirements at a single interval. For thromboelastography (TEG) and scanning electron microscopy (SEM) studies, citrated blood samples were taken, and EDTA blood was used for the complete blood profile.

A. Blood Profile

Using Haematology analyzers, the EDTA fresh blood was used for complete blood profile analysis (typically done in a Samsung H.C. 10).

B. Thromboelastography (TEG) to Measure Coagulation Parameters (Viscoelastic Properties)

To determine clot formation and clot power, TEG is usually used [15]. Whole blood and platelet-poor plasma were used in a computer-controlled TEG hemostasis system (Model 5000, Hemoscope, Niles, IL) [16]. To test the complete coagulation process, whole blood was used, and platelet-poor plasma was used to evaluate coagulation without the effect of platelets on the coagulation pathways. TEG parameters included: (1) R (reaction time, min): a calculation of the initiation of the clot from the beginning of the test to the initial formation of fibrin (amplitude of 2 mm); i.e., initiation time, (2) K (kinetics measured in minutes): time is taken to reach a certain amount of clot intensity (amplitude of 20 mm); i.e., amplification, (3) A (alpha) angle (slope between the traces represented by R and K, measured in degrees); (4) M.A. (maximum amplitude): reflects the ultimate strength of the fibrin clot; (5) MRTG (maximum rate of thrombus generation); (6) TMRTG (time to a maximum rate of thrombus generation): time interval observed before the maximum speed of the clot growth; (6) TTG (total thrombus generation): the clot strength - the amount of total resistance generated during clot formation.

### TABLE I: DEMOGRAPHIC HBA1C LEVELS, MEDICATION, AND ASPARTAME USAGE IN TYPE II DIABETES PATIENTS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gender</th>
<th>Age</th>
<th>HbA1c (%) &lt;7%</th>
<th>Dyslipidemia</th>
<th>Antihyperglycemic</th>
<th>Antihypertensive</th>
<th>Aspartame usage:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>53</td>
<td>6.7</td>
<td>Simvastatin</td>
<td>Metformin</td>
<td>Coversyl</td>
<td>2 - packets of Canderel /per day in porridge - 2 years</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>62</td>
<td>6.5</td>
<td>Metformin</td>
<td>X</td>
<td></td>
<td>5 - packets of Canderel /per day in tea &amp; porridge - 3 years</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>6.1</td>
<td>X</td>
<td>Metformin</td>
<td>X</td>
<td>2 - packets of Canderel /per day in tea - 3 years</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>67</td>
<td>6.6</td>
<td>Simvastatin</td>
<td>Metformin</td>
<td>X</td>
<td>1 - tablet of Nutra /day in tea - 3 years</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>75</td>
<td>6.4</td>
<td>Simvastatin</td>
<td>Metformin</td>
<td>X</td>
<td>2 - tablets of Nutra /day in tea - 5 years</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>72</td>
<td>6.2</td>
<td>Atorvastatin</td>
<td>Metformin</td>
<td>X</td>
<td>6 - packets of Canderel /per day in tea &amp; porridge - 9 years</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>57</td>
<td>6.3</td>
<td>Atorvastatin</td>
<td>Metformin</td>
<td>Nifedipine</td>
<td>4 - packets of Canderel /per day in tea - 3 years</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>52</td>
<td>6.5</td>
<td>Atorvastatin</td>
<td>Metformin</td>
<td>Amlodipine</td>
<td>4 - 6 packets of Canderel /per day in tea - 3 years</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>73</td>
<td>6.6</td>
<td>Atorvastatin</td>
<td>Metformin</td>
<td>X</td>
<td>3 - 5 packets of Canderel /per day in tea - 3 years</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>69</td>
<td>6.5</td>
<td>Simvastatin</td>
<td>Actraphane</td>
<td>X</td>
<td>3 - 4 tablets Nutra/day in tea - 20 years</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>62</td>
<td>6.3</td>
<td>Simvastatin</td>
<td>Metformin</td>
<td>Nifedipine</td>
<td>4 - tablets Nutra/day in tea - 10 years</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>49</td>
<td>6.4</td>
<td>Simvastatin</td>
<td>Metformin</td>
<td>X</td>
<td>4 - tablets Nutra/day in tea - 10 years</td>
</tr>
</tbody>
</table>
C. Scanning Electron Microscopy (SEM) of Platelets

To look at platelets and fibrin structure, high magnification SEM analyses was used. To create smears, dehydrated, dried, assembled, and coated with carbon according to previously mentioned methods, 20 μL of fixed whole blood and platelet-poor plasma were placed on a broad glass coverslip [10]. A Zeiss ULTRA Plus FEG-SEM was used to find the surface characteristics of the platelets, and morphologies were obtained at 1 kV.

IV. FINDING AND INTERPRETATION

There were no significant changes in the complete blood profile as shown in Table II of T2D subjects who consumed aspartame, such as haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell (RBC), white blood cell (WBC) and Platelet (PLT). In diabetic subjects, platelet counts are typically average [17], [18] and aspartame use does not significantly impact platelet counts. We also found that platelet counts were average in subjects receiving aspirin as an anticoagulant. Research by Roberts that looked at aspirin-induced thrombocytopenia found four such cases [19]. However, several studies indicate increased activation or platelet activity in T2D subjects with average platelet counts [20]-[22].

TABLE II: FULL BLOOD PROFILE IN T2D SUBJECTS CONSUMING ASPARTAME

<table>
<thead>
<tr>
<th>Blood profile (with standard range value)</th>
<th>Observed Blood profile (in average) for aspartame consumer (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (11 - 14 g/dl)</td>
<td>13.25 ± 2.09</td>
</tr>
<tr>
<td>HCT (34 - 40%)</td>
<td>38.62 ± 5.70</td>
</tr>
<tr>
<td>MCV (75 - 87 fl)</td>
<td>84.83 ± 3.37</td>
</tr>
<tr>
<td>MCH (24 - 30 pg)</td>
<td>29.90 ± 3.33</td>
</tr>
<tr>
<td>MCHC (31 - 36 g/dl)</td>
<td>34.24 ± 0.76</td>
</tr>
<tr>
<td>RBC (4.00 - 5.20 x 10^12/l)</td>
<td>4.55 ± 0.68</td>
</tr>
<tr>
<td>WBC (5.00 - 15.00 x 10^9/l)</td>
<td>8.02 ± 2.77</td>
</tr>
<tr>
<td>PLT (178 - 400 x 10^9/l)</td>
<td>246.25 ± 67.32</td>
</tr>
</tbody>
</table>

Using thromboelastography, details of which are indicated in Table III, we observed that R time is earlier in the whole blood among T2D subjects consuming aspartame, suggesting activation of more platelets leading to faster clot formation. However, the TMRTG is higher, and in platelet-poor-plasma, the M.A. is lower. Also, whole blood and platelet-poor plasma are higher in TTG.

Generally, due to contact activation, platelets display only slight formation of pseudopodia. However, more platelet activation and spreading were noted among T2D subjects consuming aspartame during SEM analysis Fig. 2 a-b. The platelet dysfunction, altered fibrin morphology, and this fibrin fiber formation showed a dense matted fibrin deposit as observed in this study is shown in Fig. 3 a-b. In a previous study, once animals were treated with 34 mg/kg of aspartame, 26 times for just two months, fibrin networks had a denser appearance with a thicker matted exemplary fiber network covering thick major fibers, and platelet aggregates were also much more granular than that of the platelet aggregates of the globular control. This animal study suggests that aspartame use, which can interfere with coagulation, can cause a delayed breakup of fibrin after blood clotting [23].

TABLE III: TEG PARAMETERS IN T2D SUBJECTS CONSUMING ASPARTAME

<table>
<thead>
<tr>
<th>TEG components (with standard Value) in whole blood</th>
<th>Observed TEG results for aspartame consumers in whole blood</th>
<th>TEG components (with standard) in platelet-poor plasma</th>
<th>Observed TEG results for aspartame consumers in platelet-poor plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (9 - 27 min)</td>
<td>7.10 ± 1.22*</td>
<td>R (5 - 11 min)</td>
<td>8.90 ± 1.42</td>
</tr>
<tr>
<td>K (2 - 9 min)</td>
<td>2.87 ± 1.19</td>
<td>K (1.5 - 4.5 min)</td>
<td>2.51 ± 2.06</td>
</tr>
<tr>
<td>Angle (22 - 58 mm)</td>
<td>53.60 ± 11.03</td>
<td>Angle (44 - 70 mm)</td>
<td>61.35 ± 13.33</td>
</tr>
<tr>
<td>MA (44 - 64 mm)</td>
<td>53.79 ± 12.59</td>
<td>MA (53 - 70 mm)</td>
<td>37.08 ± 9.72*</td>
</tr>
<tr>
<td>MRTG (0 - 10 dcs)</td>
<td>4.96 ± 2.25</td>
<td>MRTG (4 - 9 dcs)</td>
<td>7.67 ± 3.87</td>
</tr>
<tr>
<td>TMRTG (5 - 23 min)</td>
<td>9.27 ± 2.05</td>
<td>TMRTG (3 - 4 min)</td>
<td>10.59 ± 1.87*</td>
</tr>
<tr>
<td>TTG (127 - 237 dcs)</td>
<td>692.59 ± 37.08</td>
<td>TTG (127 - 237 dcs)</td>
<td>315.18 ± 1.41</td>
</tr>
</tbody>
</table>

Reaction time (R; sec) - the time elapsed until the first measurable clot forms. Clot kinetics (K; sec) - the time elapsed until the clot reaches a fixed strength (20 mm). Angle (°) reflects the speed of fibrin accumulation. Maximum amplitude (MA; mm) reflects the strength of the clot. Coagulation index (CI; dynes/sec) provides a representation of a patients coagulable state. Lys 30 (° of the lysed clot) - lysis of a clot within 30 min. Clot firmness (G; dynes/cm²) the shear elastic modulus strength. Maximum rate of thrombus generation (MRTG; Dyn cm² s⁻¹) the maximum speed of the clot growth. Time to maximum rate of thrombus generation (TMRTG; min) the time period before the maximum speed of the clot growth. Total thrombus generation (TTG; Dyn cm²) the total clot resistance. *Abnormal values.
An activated platelet demonstrates the formation of pseudopodia. The abnormal TEG parameters observed in this study could be exacerbated by platelet hyperactivation [24]. Phenylalanine is available in the human fibrinogen alpha-chain at location P9. In contrast, aspartate at location P10 plays a critical role in transforming fibrinogen to fibrin by catalyst-thrombin in the blood coagulation process [25]-[27]. Aspartame is also mainly composed of phenylalanine, including aspartate, so it could have inhibitory activity on thrombin and intervene with the coagulation mechanism [28]. The hyperactive platelets in T2D subjects enhance the release of 5-HT and increased its plasma levels. This increase in 5-HT plasma can contribute to the pathogenesis of atherosclerosis and vasospasm [29]. Approximately 90 - 95% of the body's serotonin-released serotonin and regulated platelet aggregation are mainly contained in platelets and enterochromaffin cells [30]. However, platelet activity can also be modulated by releasing CNS serotonergic neurons with 5-HT [31], [32]. Therefore, after aspartame use, decreased cerebral serotonin levels [33] may affect platelet function. The synthesis of multiple clotting factors and the weakening of fibrinolytic activity are induced by generally high circulating cortisol [34], [35]. Hypercoagulable disorders and thromboembolic complications have been linked with endogenous and exogenous hypercortisolism [36]. Aspartame is a chemical stressor associated with irregular hypothalamic-pituitary-adrenal (HPA) axis function with high plasma corticosterone or (cortisol) levels in rats [37], [38]. Corticosterone also impacts the transmission of tryptophan across the blood-brain barrier and plays a permissive function in maintaining serotoninergic neurons during stressful circumstances in the central 5-HT system [39], [40]. The use of aspartame may impact central serotoninergic neurons [33], and central serotoninergic neurons may modify platelet function [31], [32]. Therefore, this elevated cortisol may be responsible for worsening the hypercoagulable condition in T2D subjects after aspartame use. Increased oxidative stress was associated with increased platelet activation in T2D subjects [41]. Oxidative stress in the blood was caused by the oral administration of aspartame in rats [23], [42]-[46]. It may have a role in exaggerating platelet activation in diabetic patients consuming aspartame. The hypercoagulable condition and increased resistance to fibrinolysis in diabetic patients are related to an augmented risk of thrombosis [18], [47].

V. CONCLUSION

The platelet dysfunction and altered fibrin morphology observed in this study exacerbate the complexity of cardiovascular disease in T2D patients. A hypercoagulable condition could increase the risk of thromboembolic complications and an increased incidence of vascular disease. This knowledge may be used to build awareness among consumers of aspartame.

LIMITATION

Owing to time constraints and a lack of funds, the research was limited to a small number of patients, and healthy people were exempted.

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CONFLICT OF INTEREST

The authors have declared no conflict.

AUTHOR CONTRIBUTIONS

AKC was in responsibility of the study's conception and design, as well as sample preparation and data analysis. The article's first and final draughts were written by AKC. The manuscript's content and similarity index are the responsibility of all authors, who have critically examined and approved the final draught.

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