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Erythrocyte Osmotic Fragility and Malondialdehyde Concentrations of Water Truck Pushers in Samaru, Sabon-Gari Local Government Area of Kaduna State, Nigeria

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Abstract

Water vending is a significantly lucrative business laden with stressful and strenuous activities making it to be implicated in oxidative damage, lipid peroxidation and the susceptibility of erythrocytes to haemolysis. The study was designed to investigate the degree of erythrocyte osmotic fragility (EOF) and Malondialdehyde (MDA) concentrations among water vendors and non-water vendors. Copies of questionnaire were randomly distributed in the study area to a total of 192 subjects of which water truck pushers (experimental group) were 96 and non-water truck pusher (Control group) were also 96 individuals. 5ml of blood was obtained from the median cubital vein via venepuncture. Ethical clearance was obtained from the health research ethics committee of A.B.U, Zaria, Nigeria. 3ml of samples stored in EDTA bottles were used for EOF whereas serum samples obtained from 2ml of centrifuged blood were assayed biochemically for MDA concentration using TBARS assay. Data obtained were analysed using independent sample T-test and cross Tab for descriptive statistics. The result showed a significant ($P < 0.05$) increase in serum MDA concentrations of the experimental group as compared to controls with a value of 230.33 ± 4.75 compared to 215.61 ± 3.59nmols/mL respectively. EOF of water vendors showed significant ($P < 0.05$) increase with a value of 85.83 ± 0.37% when compared to 78.69 ± 0.53% for non-water truck pushers at 0.4% concentration of NaCl solution. These findings suggest that a significant increase exists in the EOF and MDA concentration of water vendors and as such the need to mitigate this effect.

Keywords: Water vendors, Oxidative stress, Lipid peroxidation, Erythrocyte osmotic fragility, Ergonomics.

Introduction

Water remains one of the cardinal requirements to sustain life demanded for both domestic and non-domestic purposes. The WHO/UNICEF describes reasonable access to water as being "the availability of at least 20 litres per person per day from a source within one kilometre of the users dwelling" (Howard and Bartram, 2003). However, one-sixth of humanity (1.1 billion people) lack access to any form of improved water andonly 61% of the sub-Saharan population have easy access to improved water sources (Nganyanyukaa*et al.,* 2014).65 million Nigerians have no access to safe water (Majuru*et al.,* 2011) and with the inadequacy of pipe-borne water being a growing problem, individuals are compelled and resort to buying water from vendors (Omalu*et al.,* 2010).

Water vendors/water truck pushersconstitute a group of distributive or ambulatory vendors also known as pushcart vendors, that supply water in jerrycans to customers at their doorsteps or at the street corner (Kjellen, 2006) using metal pushcarts designed for this purpose by local blacksmiths (Youngstedt*et al.*, 2016).They are commonly called *mai ruwa* (water men) or *mai kura* (truck pushers) in the Hausa dialect. Water vending is proving to be a rapidly expanding source of employment for individuals, associated with prolonged physical stress and physically strenuous activity (Bhowmik and Saha, 2012) due to itsdemand-driven nature.

Almost all studies on them (the world over) show that they work for long hours and under trying conditions(Bhowmik and Saha, 2012). Looking at water vending from an ergonomic and kinesiologic perspective, truck pushing involves both upper and lower body exercise. Such exercise in heat, as compared with a neutral environment, causes many physiologic changes in the dynamics of the human body and can overload the body's ability to properly respond to the imposed stress, which can result in hyperthermia, dehydration, deteriorated physical and mental performance, and a potentially serious (even fatal) exertion heat illness (Casa, 1999). Stress and Strenuous physical activity have been implicated in oxidative damage (Güreş*et al.,* 2009;

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Breitenbach and Eck, 2015), lipid peroxidation (Gwozdzinski*et al.,* 2017) and osmotic fragility (Olszewska*et al.,* 2012; Jimoh *et al.,* 2015).

Stress has been associated with the pathomechanisms of different diseases, and redox processes are believed to play a major role in its generation, defence and signaling (Breitenbach and Eck, 2015). Avloniti*et al.*(2017), exerts that exposure to strenuous activities results in marked elevations of oxidative stress. Strenuous physical activity increases oxygen consumption and increasesthe concentrations of free radicals (Sureda*et al.,* 2005), which if severe can cause damage to the cells (by inducing oxidative stress) and when mild has a regulatory function due to the activation of antioxidant enzymes, thus offeringantioxidative protection (Ďuračková, 2010).

This research evaluates the extent of erythrocyte osmotic fragility (OST) and Malondialdehyde (MDA) concentrations of water vendors in Samaru, Sabon-Gari local government area of Kaduna State, Nigeria.With the goal of getting baseline values for water vendors, to enable accurate interpretation of clinical values in the management of diseases affecting water vendors, to deepen current knowledge on mechanisms by which cells respond to strenuous occupational activity and to provide clues for further understanding strenuous occupational activities. This might also help in the evaluation of the opinion on antioxidant supplementation during exercise and strenuous physical activity.

Methods

Study design and Sample population

A case-control study of 192 randomly selected subjects (96 water vendors and 96 non-water vendors) was adopted for this evaluation. Apparently healthy water vendors with at least 1(one) year of working experience were mobilised during aone month follow-up period. Data were obtained via detailed interviews, administration of questionnaires and field experimentation. While 5ml collected blood samples were used for in-vitro experimental analysis/assay in the laboratory. The study protocol was approved by the Health Research Ethics Committee of Ahmadu Bello University Teaching Hospital (ABUTH) (ABUTH/HREC/UG/6) and all participants signed a written consent form. Subjects showing evidence of target-organ-damage were excluded from the study. An exclusion criterion for alcohol usage, pre-test exercise and prandial pre-test conditions was observed using a selection protocol consisting of clinical history, physical examination and appropriate tests.

Study location

Samaru is found in SabonGari LGA located between Ahmadu Bello University, Basawa and Bomo. It is a growing urban settlement within Zaria located approximately between latitudes 110 10` and 11011` N of the Greenwich Meridian and longitude 70 37’ and 70 40’ E of the Equator in the fringes of the Northern Guinea Savannah (Fig. 1) and having an estimated population of 343,000. It is a combination of residential and commercial areas, with many ethnic groups, major ones include Hausa, Fulani, Yoruba, Igbo, Ebira, Idoma*etcetera*. There is a strong presence of educational institutions in the district with primary, secondary as well as tertiary institutions located in the area (Dzikwi*et al.,* 2012).

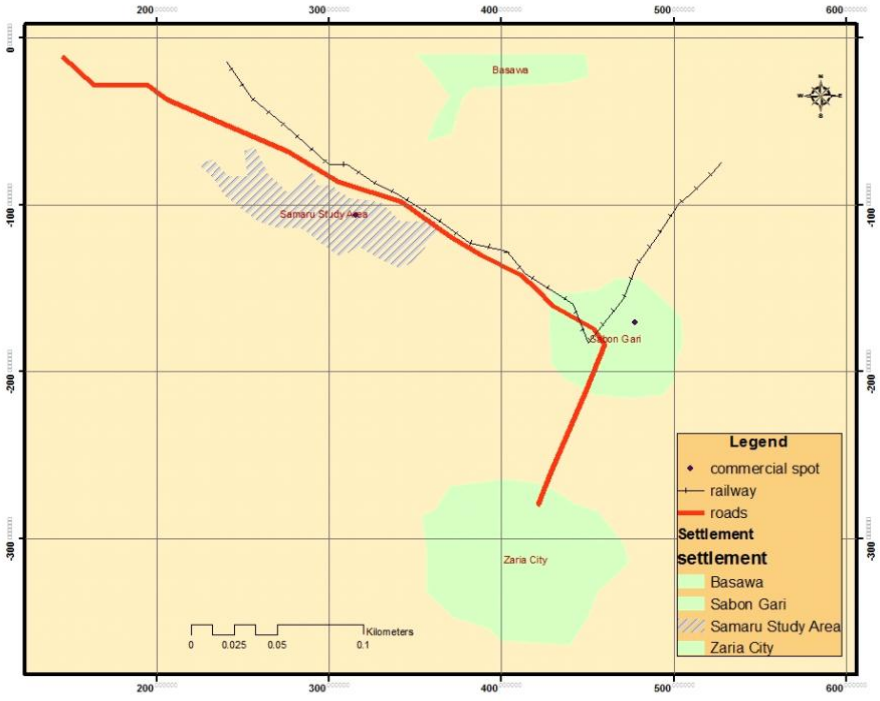

Figure I
Sabon-Gari showing Samaru (Adopted from Benedine*et al.,* 2011).

Experimental procedure and Blood analysis

The subjects were introduced to completion of questionnaire andthe average weights of trucks and jerry cans were obtained. Measurements were done using Suspended weighing balance (Model 235, TL Weigh, 200kg × 1kg, Made in England) at Badamasi Iron Store, Daraka, Samaru. 150microliter (0.15ml) of serum samples obtained from centrifuged (Bench Centrifuge, Batch No. 68/3.856/253, Baird and Tatlock Ltd., England)blood were used to determine the serum MDA levelvia Thiobarbituric Acid Reactive Substance (TBARS) assay using the modified method of Niehaus and Samuelsson (1968), as described by Akanji*et al.*(2009). The resulting pink solutions were then transferred into cuvettes and placed in a spectrophotometer (Spectrumlab 22PC, Batch No. 22PC08211) to determine the absorbance at wavelength of 535nm and the malondialdehyde formed was then calculated using the molar extinction coefficient of 1.56×10^{-5} $cm^{-1}M^{-1}$**.** MDA concentration= absorbance of sample/ 1.56×10^{-5} $cm^{-1}M^{-1}$.

The erythrocyte osmotic fragility was determined using the method described byBaker *et al.* (2002). 0.05ml of anticoagulated blood (in EDTA bottles) was added into 5ml of solutions with decreasing strengths of hypotonic saline. Gently mixed and allowed to stand for 5 mins and thecentrifuged for 3 minutes.The supernatant solution was read using a colorimeter (Optima, Japan) at a wavelength of 540nm. The percentage haemolysis was calculated using the formula described by Faulkner and King (1970): (Optical density of test / Optical density of distilled water) x 100 = Percent haemolysis of each sample at different saline concentration.

Statistical analysis

An Independent samples T-test was conducted to obtain the mean ± the standard error of mean (SEM) while Cross tabulations were used as inferential non-parametric test The analysis was carried out using Statistical Package for Social Sciences (SPSS software version 20; SPSS Inc., Chicago) and Microsoft Excel ©2010. Values of $P< 0.05$ were considered significant.

Results

Effect of water vending on the erythrocyte osmotic fragility

The experimental group of water truck pushers show an extremely significant ($P < 0.001$) increase in the degree of haemolysis compared to the control group of non-water truck pushers with average values of 39.40 ± 0.65% haemolysis for experimental group and 31.81 ± 0.39% haemolysis for the controls at 0.7% NaCl Concentration, 52.56 ± 0.66% haemolysis for the experimental group and 47.04 ± 0.44% haemolysis for the control group at 0.6% NaCl Concentration, 72.81 ± 0.65% haemolysis for the experimental group and 62.67 ± 0.53% haemolysis for the control group at 0.5% NaCl Concentration, 85.83 ± 0.37% haemolysis for the experimental group and 78.69 ± 0.53% haemolysis for the control group at 0.4% NaCl Concentration and 99.33 ± 0.17% haemolysis for the experimental group and 93.67 ± 0.39% haemolysis for the control group at 0.3% NaCl Concentration respectively. Complete haemolysis (100.00 ± 0.00%) was observed for both groups at 0.2% NaCl Concentration. No significant degree of haemolysis ($P > 0.05$) was observed between the groups at 0.9% NaCl Concentration.

Table 1
Result from Osmotic Fragility Test for water vendors and non-water vendors

Group	0.9% NS	0.7% NS	0.6% NS	0.5% NS	0.4% NS	0.3% NS	0.2% NS
Non-Water Vendors	0.58 ± 0.29	31.81 ± 0.39	47.04 ± 0.44	62.67 ± 0.53	78.69 ± 0.53	93.67 ± 0.39	100.00 ± 0.00
Water Vendors	0.00 ± 0.00	39.40 ± 0.65*	52.56 ± 0.66*	72.81 ± 0.65*	85.83 ± 0.37*	99.33 ± 0.17*	100.00 ± 0.00
P value	0.155	0.000	0.000	0.000	0.000	0.000	-

* = indicates significance ($P< 0.05$) in haemolysis of water vendors as compared to controls (non-water vendors).

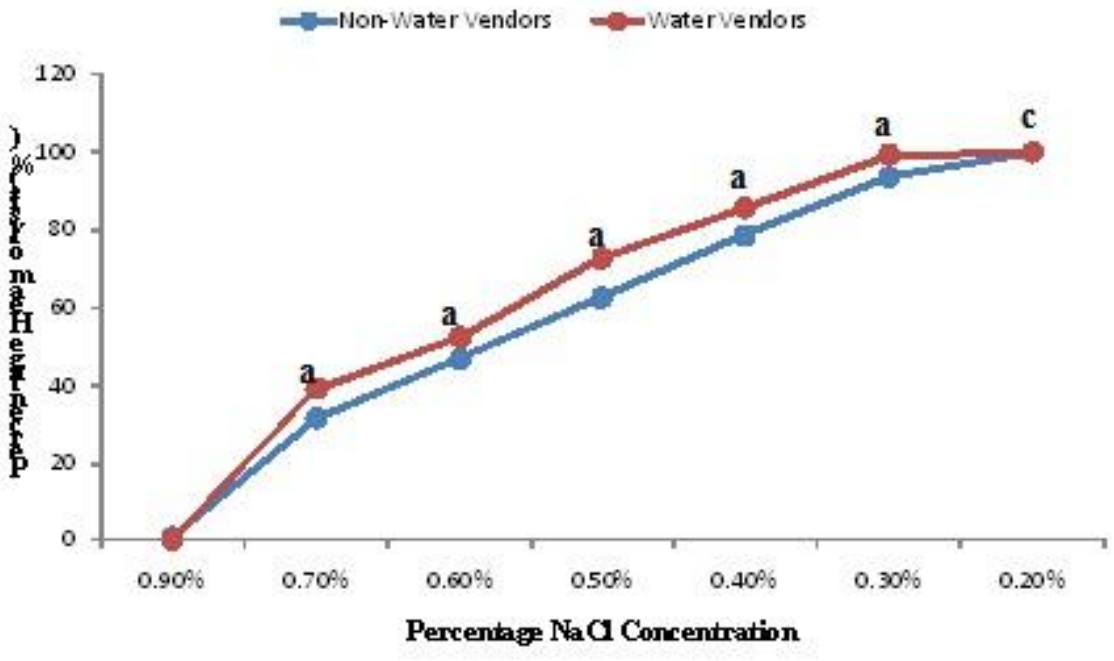

Figure II
Erythrocyte osmotic fragility curve for the experimental and control groups.Superscript letter [a] indicates significant ($P< 0.05$) haemolysis in water vendors as compared to controls (non-water vendors). Superscript [c]indicates complete haemolysis.

Effect of water vending on malondialdehyde concentrations

The experimental group (water truck pushers) show a very highly significant ($P< 0.001$) increase in the serum concentration of malondialdehyde with a value of 230.33 ± 9.57nmols/ml of plasma as compared to the control group (non-water truck pushers) with a value of 215.61 ± 7.24nmols/ml of plasma, respectively.

Table 2
Result obtained from Independent Samples Test for Malondialdehyde levels

Group	MDA (nmols/ml)
Non-Water Vendors	215.61 ± 3.59
Water Vendors	230.33 ± 4.75*
P value	0.000

* = indicates significance ($P< 0.05$) of MDA concentrations in water vendors as compared to controls (non-water vendors).Descriptive statistics for Malondialdehyde concentrations: from Cross-tabulation [X^2 (Pearson Chi-Square) = 180.000, df = 90].

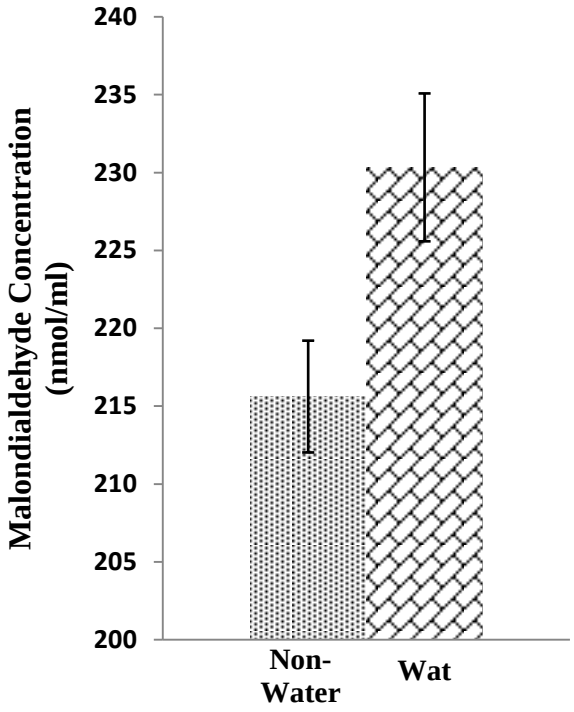

Figure III
Showing the average serum Malondialdehyde concentrations for water vendors and non-water vendors. Different Superscript letters [a, b] indicate significant ($P< 0.05$) difference between groups.

Result from field measurements

The average dry weight of one truck was found to be 47kg, while the average weight of one empty 25 liter water jerrycan was also measured to be 1kg and average weight of a 25 literjerrycan full of water was 25kg.

Result of Questionnaire Study

After collation of survey questionnaires, data entryand analysis were performed and a frequencytable was developed for the sample population. As seen below.

Table 3
Showing some responses of water vendors in percentage scale

S/N	Question	Response	Percentage (%)
1	Have you ever suffered from any joint pain as a result of your work?	Yes	60.4
		No	39.6
2	Have you ever suffered from any other ailment due to your job?	Yes	54
		No	42
3	Does your job affect your health negatively?	Yes	50
		No	46
4	How long does it take you to sell one truck of water?	Within an hour	66.7
		1-2hours	31.3
		2-3 hours	2.1
5	What is the daily time duration of your work?	1-3 hours	2.1
		4-6 hours	10.4
		7-9 hours	12.5
		10-12 hours	58.3
		13-15 hours	16.7
6	How many containers do you operate with?	6-10 Jerry cans	2.1
		11-15 Jerry cans	83.3
		16-20 Jerry cans	8.3
		21-25 Jerry cans	2.1
		26-30 Jerry cans	2.1

7	**What number of trucks do you sell in a day?**	**1-3 trucks**	2.1
		4-6 trucks	39.6
		7-9 trucks	47.9
		10-12 trucks	8.3
8	**Is there ever a period in which you do not work?**	**Yes**	81.3
		No	18.8

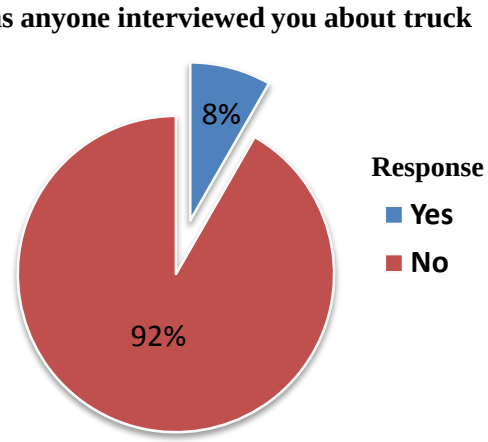

Figure IV
Pie chart showing response to the questionnaire

Discussion

Result from osmotic fragility of water vendors indicate a significant ($P< 0.05$) increase in erythrocyte haemolysis as compared to that of non-water vendors.This result is in agreement with research conducted and described by Gures*et al.* (2009), who reported a significant increase in the erythrocyte osmotic fragility of medium level trained athletes, one hour after submaximal exercise (i.e. after running 5000metres in 16minutes) and Ayo *et al.* (2015), who reported increased haemolysis in rabbits subjected to road transportation (for 2 h under hot-humid climate). The increased erythrocyte osmotic fragility as observed in the present study implies that water vending increases haemolysis which is evident from the strenuous occupational activity. This agrees with the findings ofOlaifa*et al.* (2012), who showed that physical exertion increases haemolysisin donkeys and Adah*et al.* (2016), reported a significant increase in the erythrocyte osmotic fragility of one-humped camels subjected to packing and trekking, with bags grains of 200 kg (which are half the body weight of each camel) for 10km. This further confirms the theory that exposure to oxidative stress increases haemolysis due to the altered mechanical properties of erythrocytes, as reported by Jimoh *et al.* (2015).A single bout of strenuous activity often leads to an acute oxidative stress, which may result in an increased level of oxidized molecules and macromolecules (Powers *et al.*, 2011). However, factors such as intensity, duration and the subject's individual (genetic and physical) characteristics may impact the degree of oxidation (Gwozdzinski*et al.*,2017).

This phenomenon of increased membrane fragility could probably result from the extensive degree of strenuous physical activity associated with vending which triggers the development of oxidant species. Increased oxidized glutathione levels occasioned by the deficiency of reduced glutathione antioxidant in the pentose phosphate pathway/hexose monophosphate shunt could be triggered by insufficient nutrient substrate (*Id est.* glucose). Apparently, the oxidized glutathione (an oxidant) then acts by degrading membrane proteins and producing increasingdegree membrane lipid peroxidization, an underlying cause for haemolysis (Sastre*et al.*, 1992; Ďuračková, 2010). Thus, upon further oxidative or osmotic stress, the membranes of erythrocytes lose their resilience and lyse, releasing their contents, which is then evident in serum as an increased optical density/absorption. Lipid peroxidation in the erythrocytes membrane could also result from increased production of superoxide from the respiratory chain and from membrane xanthine oxidase, which is triggered by increased consumption of oxygen during physical activity (Davies *et al.*, 1982;Sastre*et al.*,1992; Reid *et al.*, 1993; Gómez-Cabrera *et al.*, 2003; Pattwell*et al.*, 2004). Research byViña*et al.* (2006), demonstrated that the oxidative stress was reduced in cyclers who were taking the xanthine oxidase inhibitor allopurinol.

The extensive degree of strenuous physical activity associated with water vending may be a consequence of the fact that most water vendors (83.3%) carry between 11-15 of 25 literjerry cans, 7-9 times a day, for an average period of about 10-12 hours per day (from dawn till dusk),. This is usually along bad roads that are difficult to trespass and for long distances. Given that each jerry can of water (25 liter) weighs about 25kg,

a vendor (averagely weighing 70kg) carrying 14 jerry cans will be pushing on average 350kg of water (14×25kg) plus an additional truck weight of 47kg; giving a total net weight of approximately 400kg (about 6 times his weight) from probably 7am to 6pm (11hrs). The increased demand for their services is another likely contributor, with 66.7% reportedly selling a truck of water within the hour of procurement.

Increase in the serum malondialdehyde concentration of water vendors as compared to non-water vendors is indicative of an increased lipid peroxidation in the erythrocyte membrane of water vendors and is confirmed by the increased osmotic fragility in our result. This finding is in consonance with report by Cingi*et al.* 2012, who illustrated that the occurrence of oxidative stress is exemplified by increased MDA concentration.Adah*et al.* (2016), obtained a significant increase in theserum malondialdehyde concentration of camels subjected to packing. This finding also supports the earlier observation that increased MDA values can be due to stress (Belge*et al.*, 2003).

The rise in MDA concentrations obtained in the present study suggests that lipid peroxidation is also initiated by strenuous activity.This effect was confirmed in the research by Olaifa*et al.* (2012), which showed that the serum MDA concentration increased significantly in donkeys subjected to physical exertion. Adenkola*et al.* (2016), also demonstrated a gradual rise in MDA concentrations of Rams subjected to the strain of road transport.

The rise in MDA concentrations obtained in the present study, is possibly due to the fact that lipid peroxidation, a well-established mechanism of cellular injury and a useful indirect indicator of oxidative stress(Jetawattana, 2005; Shohag*et al.*, 2012) can be initiated by any chemical species such as oxidized glutathione which extracts a hydrogen atom from the side chain double bonds of a polyunsaturated fatty acid (PUFA) which is generally present in the cell membranes (Singh *et al.*, 2014). The probably increased oxidized glutathione level is an outcome from strenuous activity which results in the formation of lipid peroxides which are unstable compounds (Grotto *et al.*, 2009) that degrade rapidly into a variety of sub products, such as MDA (Mateos*et al.*, 2005). Arachidonic acid is the polyunsaturated omega-6 fatty acid (a PUFA) present in the cell membranes, brain, muscles and liver that contains many uninterrupted methylene double bonds that serve as a source of hydrogen atoms for the free radicals (Singh *et al.*, 2014).

The present study also shows that over half of the water vendors have suffered from joint pains and other ailments due to their profession. This might be a consequence of the deteriorative effect of oxidative stress (Lushchak, 2014) and emphasizes the need to mitigate these effects. This research shows great potential as about 91.7% of vendors report to have never been interviewed and examined with regards to water vending.

Conclusion

In conclusion, the present study highlights observation that the oxidative and fragility parameters are significantly compromised during water vending in Samaru, North-western Nigeria. This is evident from the increased malondialdehyde concentration and high tendency to haemolysis of the red cell membrane in water truck pushers as compared to non-water truck pushers. Thus, drawing emphasis for the need to ameliorate the possible outcomes.

References

1. Aboh, E. A., Giwa, F. J. and Giwa, A. (2015).Microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria.*Annals of African Medicine,* 14: 32-38.
2. Adah, A. S., Ayo, J. O., Rekwot, P. I., Aluwong, T. and Arimie, D. I. (2016). Erythrocyte osmotic fragility and serum malondialdehyde concentration in the one humped camel (*camelusdromedarius*) subjected to packing (load-carrying) in the semiarid zone of Nigeria.*Alexandria Journal of Veterinary Sciences*, 48(1): 93-98.
3. Adenkola, A. Y, Adah, A. S. and Ambali, S. F. (2016).The effects of vitamins C and E on erythrocyte osmotic fragility, serum malondialdehyde concentrations and surface erythrocyte sialic acid in rams following road transportation.*Alexandria Journal of Veterinary Sciences*, 48(2): 9-17.
4. Adeoye, P. A., Adeolu, A. R. and Ibrahim, H. M. (2013). Appraisal of rural water supply: case study of Kwara state, north-central Nigeria. *International Journal of Basic and Applied Science*, 1(4): 816-826.
5. Akanji, M. A., Adeyemi, O. S., Oguntoye, S. O. and Suleiman, F. (2009).Psidiumguavaja extract reduces trypanosomiasis associated lipid peroxidation and raised glutathione concentrations in infected animals. *Experimental and Clinical Sciences International Journal*, 8: 148-154.
6. Alessio, H. M. (2000). Oxidative stress: mechanisms and manifestations. in*Handbook of oxidants and antioxidants in exercise*; Sen, C. K., Packer, L. and Hanninen, O., 1st ed.; Elsevier: Amsterdam, Pp. 797-829.
7. Anila, L. and Vijayalakshmi, N. R. (2003).Antioxidant action of flavonoids from Magnifiers indica and Emblicaofficinalis in hypercholesterolaemic rats.*Food Chemistry*, 83(4): 569-579.
8. Antonelou, M. H., Papassideri, I. S., Karababa, F. J., Stravopodis, D. J., Loutradi, A. and Margaritis, L. H. (2003).Defective organization of the erythroid cell membrane in a novel case

of congenital anemia.*Blood Cells, Molecules and Diseases,*30: 43-54.

9. Avloniti, A., Chatzinikolaou, A., Deli, C. K., Vlachopoulos, D., Gracia-Marco, L., Leontsini, D., Draganidis, D., Jamurtas, A. Z., Mastorakos, G. and Fatouros, I. J. (2017). Exercise-induced oxidative stress responses in the pediatric population. *Antioxidants*, 6(6): 1-16.
10. Ayo, J. O., Minka, N. S. and Hussein, K. A. (2015). Effects of ascorbic acid administration on erythrocyte osmotic fragility in rabbits (*Oryctolaguscuniculus*) subjected to road transportation. *Journal of Applied Animal Research*, 43(1): 26-32.
11. Baker, F. J., Silverton, R. E., Pallister, C. J., Hornby, A., Luxton, R. W. and Griffin, R. L. (2002). Tests for erythrocyte sedimentation and fragility. *Introduction to medical laboratory science*, (5th ed.). Butterworth-Heinemann: London. Pp. 101-110.
12. Belge, F., Cinar, A. and Selcuk, M. (2003).Effects of stress produced by adrenocorticotropin on lipid peroxidation and some antioxidants in vitamin C treated and nontreated chickens.*South African Journal of Animal Science*, 33(3): 201-205.
13. Benedine, A., Robert, T. A. and Abbas, I. I. (2011). The impact of spatial distribution of solid waste dumps on infrastructure in Samaru, Zaria, Kaduna State, Nigeria using geographic information systems (GIS). *Research Journal of Information Technology*, 3(3): 113-117.
14. Bhowmik, S. K. and Saha, D. (2012).Street vending in ten cities in India.*National Association of Street Vendors of India*, Pp. 1-174.
15. Bickford, M. (2005). Stress in the workplace: a general overview of the causes, the effects, and the solutions. *Canadian Mental Health Association Newfoundland and Labrador Division*, Pp. 1-44.
16. Breitenbach, M. and Eck, P. (2015).Introduction to Oxidative Stress in Biomedical and Biological Research.*Biomolecules*, 5(2): 1169-1177.
17. Casa, D. J. (1999). Exercise in the heat. I. Fundamentals of thermal physiology, performance implications, and dehydration. *Journal of Athletic Training*, 34(3): 246-252.
18. Chikezie, P. C., Uwakwe, A. A. and Monago, C. C. (2010). Comparative osmotic fragility of three erythrocyte genotypes (HbAA, HbAS and HbSS) of male participants administered with five antimalarial drugs. *African Journal of Biochemistry Research*, 4(3): 57-64.
19. Cingi, C. C., Utuk, A. E., Karafakioglu, Y. S., Balkaya, I., Piskin, F. C. and Fidan, A. F. (2012). Serum lipid and protein oxidation and antioxidant status in horses naturally infected with Theileriaequi. *Revue de Médecine Vétérinaire,*163(4): 183-186.
20. Davies, K. J., Quintanilha, A. T., Brooks, G. A. and Packer, L. (1982).Free radicals and tissue damage produced by exercise.*Biochemistry and Biophysics Research Community*, 107(4): 1198-1205.
21. Delaunay, J. (2002). Molecular basis of red cell membrane disorders.*ActaHaematologica,*108(4): 210-218.
22. Delaunay, J., Alloisio, N., Morle, L., Baklouti, F., Venezia, D. N., Maillet, P. and Wilmotte, R. (1996).Molecular genetics of hereditary elliptocytosis and hereditary spherocytosis.*Annales De Genetique,*39(4): 209-221.
23. Draper, H. H., Csallany, A. S. and Hadley, M. (2000).Urinary aldehydes as indicators of lipid peroxidation in vivo.*Free Radical Biology and Medicine*, 29(11): 1071-1077.
24. Ďuračková, Z. (2007). Oxidants, antioxidants and redox stress. In: *The activity of natural compounds in diseases prevention and therapy*. Ďuračková, Z. and Knasmüller, S. (eds), SAP: Bratislava, Pp. 11-59.
25. Ďuračková, Z. (2008).Oxidants, antioxidants and oxidative stress. In: *Mitochondrial medicine. mitochondrial metabolism, diseases, diagnosis and therapy*. Gvozdjáková, A. (ed), Springer: Amsterdam, Pp. 19-49.
26. Ďuračková, Z. (2010).some current insights into oxidative stress. *Physioogical Research*, 59: 459-469.
27. Dzikwi, A. A., Ibrahim, A. S. and Umoh, J. U. (2012). Knowledge and practice about rabies among children receiving formal and informal education in Samaru, Zaria, Nigeria. *Global Journal* of *Health Science*, 4(5): 132-139.
28. Eng, J. (2003). Sample Size Estimation: How many individuals should be studied?.*Radiology*, 227(2): 309-313.
29. Everly, G. S. and Lating, J. M. (2013).The anatomy and physiology of the human stress response.*A Clinical Guide to the Treatment of the Human Stress response*, New York: Springer. Pp. 17-51.
30. Faulkner, W. R. and King, J. W. (1970).Manual of clinical laboratory procedures.published by the chemical rubber company, Cleveland, Ohio. Pp. 354.
31. Gillham, B., Papachristodoulou, D. K. and Thomas, J. H. (1997). Free radicals in health and disease, chapter 33, In: Will's: *Biochemical basis of medicine*, (3rd ed.),Butterworth-Heinemann: Oxford, Pp. 343.
32. Gómez-Cabrera, M. C., Pallardó, F. V., Sastre, J., Viña, J. and García-del-Moral, L. (2003). Allopurinol and markers of muscle damage among participants in the Tour de France.

Journal of the American Medical Association, 289(19): 2503-2504.
33. Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Garcia, G. S. S. C., Pomblum, V. J., Rocha, J. B. C. and Farina, M. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification.*Quimica Nova*, 32(1): 169-174.
34. Güney, Y., Bilgihan, A., Ciftçi, T. U., Çimen, F. and Coşkun, O. (2004).Serum malondialdehyde levels and superoxide dismutase activities in pulmonary tuberculosis and lung cancers.*Ankara ÜniversitesiDikimeviSağlıkHizmetleriMeslekYüksekokuluDergisi*, 6(2): 33-38.
35. Güreş, A., Güreş, Ş., Özdemir, N., Karul, A. B., Kozacı, D., Altun, Ç. and Gürel, G. (2009). Does submaximal exercise affect erythrocyte osmotic fragility and serum MDA levels of distance runners.*SporHekimliğiDergisiCilt*, 44: 125-130.
36. Guyton, A. C. and Hall, J. E. (2016). Contraction of skeletal muscle, *Textbook of Medical Physiology* (3rd ed.) Elsevier, Inc.; Philadelphia. Pp. 75-88.
37. Gwozdzinski, K., Pieniazek, A., Tabaczar, S., Jegier, A. and Brzeszczynska, J. (2017).Investigation of oxidative stress parameters in different lifespan erythrocyte fractions in young untrained men after acute exercise.*Experimental Physiology*, 102(2): 190-201.
38. Halliwell, B. (2007). Oxidative stress and cancer: have we moved forward. *Biochemistry Journal*, 401(1): 1-11.
39. Halliwell, B. and Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean. *British Journal of Pharmacology*, 142(2): 231-255.
40. Howard, G. and Bartram, J. (2003).Domestic water quantity, service, level and health.*World Health Organization*, Pp. 1-33.
41. Janero, D. R. (1990).Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury.*Free Radical Biology and Medicine*, 9(6): 515-540.
42. Jetawattana, S. (2005).Malondialdehyde (MDA), a lipid oxidation product. *Free Radicals in Biology and Medicine*, 77(222): 1-10.
43. Jimoh, A., Tanko, Y., Ahmed, A., Mohammed, A. and Ayo, J. O. (2015).Protective effect of resveratrol co-administration with cholesterol diet on erythrocyte osmotic fragility and malondialdehyde concentration in rabbits.*British Journal of Pharmaceutical Research*, 6(1): 14-21.
44. Jollow, D. J. and McMillan, D. C. (2001).Oxidative stress, glucose-6-phosphate dehydrogenase and the red cell.*Advances in Experimental Medicine and Biology*, 500: 595-605.
45. Khurana, I. (2012). Red Blood Cells and Anaemia, *Medical Physiology for Undergraduate Students*. (1st ed.). New Delhi: Elsevier. Pp. 100-120.
46. Kjellen, M. (2006).Water vending and the privatization of water distribution.from public pipes to private hands: water access and distribution in Dar esSalaam, Tanzania (1st ed.). Stockholm University Press: Sweden. Pp. 145.
47. Knight, J. A., Pieper, R. K. and McClellan, L. (1988). Specificity of the thiobarbituric acid reaction: its use in studies of lipid peroxidation. *ClinicalChemistry*, 34(12): 2433-2438.
48. Lefevre, G., Bonneau, C., Rahma, S., Chanu, B., Brault, D., Couderc, R. and Etienne, J. (1996). Determination of plasma protein-bound malondialdehyde by derivative spectrophotometry.*European journal of clinical chemistry and clinical biochemistry*, 34(8): 631-636.
49. Liu, J., Yeo, H. C., Doniger, S. J. and Ames, B. N. (1997). Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid). *Analytical Biochemistry*, 245(2): 161-166.
50. Lushchak, V. I. (2014).Free radicals, reactive oxygen species, oxidative stress and its classification.*Chemico-Biological Interactions*,224(3): 164-175.
51. Manevaa, A., Talevaa, B. and Maneva, L. (2003).Lactoferrin-protector against oxidative stress and regulator of glycolysis in human erythrocytes.*ZeitschriftfürNaturforschung*, 58(3-4): 256-262.
52. Margetis, P., Antonelou, M., Karababa, F., Loutradi, A., Margaritis, L. and Papassideri, I. (2007).Cells physiologically important secondary modifications of red cell membrane in hereditary spherocytosis-evidence for in vivo oxidation and lipid rafts protein variations.*Blood Cells, Molecules and Diseases*, 38(3): 210-220.
53. Mateos, R., Lecumberri, E., Ramos, S., Goya, L. and Bravo, L. (2005).Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress.Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits.*Journal of Chromatography B*, 827: 76-82.
54. National Population Commission (2007).Report of Nigeria's national population commission on

the 2006 census.*Population and Development Review*, 33(1): 206-210.
55. Nganyanyukaa, K, Martineza, J., Wesselinka, A., Lungob, J. H. and Georgiadou, Y. (2014). Accessing water services in Dar es Salaam: Are we counting what counts?.*Habitat International*, 44: 358-366.
56. Niehaus, W. G. and Samuelsson, B. (1968).Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation.*European Journal of Biochemistry*, 6(1): 126-130.
57. Nielsen, F., Mikkelsen, B. B., Nielsen, J. B., Andersen, H. R. and Grandjean, P. (1997). Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry*, 43(7): 1209-14.
58. Occupational safety and Health administration (2000). Ergonomics: The study of work. *United States Department of Labor*. Pp. 1-11.
59. Olaifa, F., Ayo, J. O., Ambali, S. F. and Rekwot, P. I. (2012). Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid. *Onderstepoort Journal of Veterinary Research*, 79(1): 1-5.
60. Olszewska, M., Wiatrow, J., Bober, J., Stachowska, E., Gołembiewska, E., Jakubowska, K., Stańczyk-Dunaj, M. and Pietrzak-Nowacka, M. (2012). Oxidative stress modulates the organization of erythrocyte membrane cytoskeleton. *Advances in Hygiene and Experimental Medicine*, 66: 534-542.
61. Omalu, I. C. J., Eze, G. C., Olayemi, I. K., Gbesi, S., Adeniran, L. A., Ayanwale, A. V., Mohammed, A. Z. and Chukwuemeka, V. (2010). Contamination of sachet water in nigeria: assessment and health impact. *Journal of Health and Allied Sciences*, 9(4): 1-3.
62. Pandolfi, P. P., Sonati, F., Rivi, R., Mason, P., Grosveld, F. and Luzzatto, L. (1995). Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defence against oxidative stress. *European Molecular Biology Organization Journal*,14(21): 5209-5215.
63. Pattwell, D. M., McArdle, A., Morgan. J. E., Patridge, T. A. and Jackson, M. J. (2004). Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells. *Free Radicals in Biology and Medicine*, 37(7): 1064-1072.
64. Powers, S. K., Nelson, W. B. and Hudson, M. B. (2011). Exercise-induced oxidative stress in humans: cause and consequences. *Free Radicals in Biology and Medicine,* 51: 942-950.
65. Reid, M. B., Khawli, F. A. and Moody, M. R. (1993).Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle. *Journal of Applied Physiology*, 75(3): 1081-1087.
66. Rybicki, A. C., Heath, R., Lubin, B. and Schwartz, R. S. (1988). Human erythrocyte protein 4.1 is a phosphatidylserine binding protein. *Journal of Clinical Investigation*, 81(1): 255-260.
67. Sastre, J., Asensi, M., Gasco, E., Pallardo, F. V., Ferrero, J. A., Furukawa, T. and Viña, J. (1992). Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. *American Journal of Physiology*, 263(2): 992-995.
68. Shohag, M. H., Ullah, M. A., Azad, M. A., Islam, M. S., Qusar, S., Shahid, S. F. and Hasnat, A. (2012). Serum antioxidant vitamins and malondialdehyde levels in patients with obsessive-compulsive disorder.*German Journal of Psychiatry*, 15(1): 10-14.
69. Singh, Z., Karthigesu, I. P., Singh, P. and Kaur, R. (2014). Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. *Iranian Journal of Public Health*, 43(3): 7-16.
70. Sureda, A., Tauler, P., Aguilo, A., Cases, N., Fuentespina, E., Co´rdova, A., Tur, J. A. and Pons, A. (2005).Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise.*Free Radical Research*, 39(12): 1317-1324.
71. Uchendu, C., Ambali, S. F., Ayo, J. O., King, A. N. E. and Angela, J. U. (2014).Erythrocyte osmotic fragility and lipid peroxidation following chronic co-exposure of rats to chlorpyrifos and deltamethrin, and the beneficial effect of alpha-lipoic acid.*Toxicology Report*, Pp. 1-17.
72. Viña, J., Borras, C., Gomez-Cabrera, M. C. and Orr, W. C. (2006). Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto) oestrogens in the modulation of adaptive response to stress.*Free Radicals in Research*, 40: 111-119.
73. Whittington, D., Laura, D. T., Okun, D. A. and Mu, X. (1989). Water vending activities in developing countries: A case study of Ukunda, Kenya. *Water Resources Development*, 5(3): 158-168.
74. Wong, S. H. Y., Knight, J. A., Hopfer, S. M., Zaharia, O., Leach, C. N. and Sunderman, F. W. (1987). Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry*, 33(2): 214-220.

75. Yagi, K., Nishigaki, I. and Ohama, H. (1968).Measurement of serum TBA-value.*Vitamins,* 37: 105-112.
76. Yakubu, S. (2013).Assessment of water quality of hand-dug wells in Zaria LGA of Kaduna State, Nigeria.*The International Journal of Engineering and Science (IJES)*, 2(11): 1-5.
77. Yawata, Y. and Jacob, H. S. (1975). Abnormal red cell metabolism in patients with chronic uremia: nature of the defect and its persistence despite adequate haemodialysis. *Blood*, 2: 231-239.
78. Youngstedt, S. M., Keough, S. B. and Idrissa, C. (2016). Water vendors in Niamey: considering the economic and symbolic nature of water. *African Studies Quarterly*, 16(2): 27-46.