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SEMINAL PLASMA LEVELS OF LEAD AND MERCURY IN INFERTILE MALES IN BENIN CITY, NIGERIA

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ABSTRACT

Background/objectives: Studies on environmental exposure to toxic metals and their effects on male reproductive function are scare in our setting. This study evaluates the levels of lead and mercury in seminal plasma of infertile males who are non-occupationally exposed in Benin City, Nigeria and to determine the relationship between seminal quality and these toxic metals. **Materials and Methods**: A total of 80 subjects participated in this study which includes 60 infertile males on routine visit to the infertility clinics in Benin City and 20 fertile males as controls. The concentration of lead in seminal plasma was assayed by atomic absorption spectrophotometer while the concentration of mercury was measured using inductively coupled plasma Mass spectrometry. Semen analyses were performed using standard techniques as recommended by World Health Organization. **Results**: Mean seminal plasma lead and mercury levels were significantly higher ($p<0.001$) in infertile males compared with controls. Mercury and lead correlated negatively ($p<0.001$) with sperm count, progressive motility, total motility and morphology but not with semen volume. There was no significant correlation between toxic metals and sperm indices in fertile males (controls). **Conclusion**: The levels of the studied toxic metals were higher in seminal plasma of infertile males and appear to have adverse effect on seminal indices in non -occupationally exposed males.

INTRODUCTION

There has been increasing concern regarding the decline in semen quality in the general population. Decline in sperm quality has been reported in both developed and developing countries including Nigeria[1-2]. Environmental factors such as exposure to oestrogen was linked to decrease in fertility in Europe [3] as well as increase prevalence of cryptorchidism and testicular cancer[4].

Occupational exposure to toxic metals such as lead and mercury has been suggested to negatively impact on sperm quality and infertility [5-7]. Toxic metals have also been reported to impair testicular function and sperm secretion in experimental animals[8]. Exposure to these toxic metals at low concentration could occur either voluntarily through supplementation or involuntarily through diet (eating of contaminated food, water) or contact with soil dust or air[9]. Even though not much study have been done regarding the effects of toxic metals on reproductive health in Nigeria, there appears to be growing concern for adverse reproductive health effects linked to low-level exposures experienced in the environment. Toxic metals could negatively impact the male reproductive system; either by disruption of hypothalamic-pituitary axis or by directly impacting adversely on spermatogenesis, resulting in poor semen quality which is associated to male infertility [10]. Studies have shown that toxic metal levels in blood may be inadequate to reveal their accumulation in the male reproductive tract [11-12]. Therefore levels of heavy metals in seminal plasma may provide a better index of exposure and effect in reproduction. This study was designed to evaluate the levels of lead and mercury in seminal plasma of infertile males in Benin City, Nigeria without occupational exposure and to correlate their levels with semen parameters.

MATERIALS AND METHODS

Type of study: Analytical study

Locus of study: The study was conducted at the Department of Medical Laboratory Science, School of Basic Medical Science, College of Medical Sciences, University of Benin, Nigeria.

Ethical approval: The study was approved by the Research Ethics committee of the University and informed consent was given by the individual subjects.

Sample size: A total of 80 participants were enrolled in the study. Sample size was determined using sample size determination in health studies 'formula and a prevalence of 10% (Lwange and Lemeshow [13].

Inclusion criteria: All consecutive subjects aged 18-50 years who had history of infertility for more than year, sperm count <20x106 cells/mL and few or no leukocyte count per field were recruited. Individuals with no history of infertility and normal semen analysis, with at least 50% motility and >30% normal sperm morphology and count of ≥20 x 106cells/mL were recruited as controls.

Exclusion criteria: Those with other specific genital and systemic disease such as genital infection, undecided testis, hepatic, renal, endocrine, autoimmune that may impair the reproductive capacity were excluded. Subjects with history of toxic metals exposure because they resided in areas known to have toxic metals contamination and cigarette smokers were also excluded from this study.

Collection of semen sample: Semen was obtained by masturbation after five days[10] of abstinence into universal container ensuring that the sperm rich first part was not lost. The sample was left on the bench at 37 º C for 30 minutes for liquefaction after which semen analysis was done according to WHO standard13.Thereafter the sample was spun at 12000g for 20 minutes to obtain seminal plasma. The seminal plasma was stored at -20ºC prior to toxic metal analysis between January and June 2014. Demographic and clinical examination findings were obtained using structured questionnaires

Semen analysis : Routine semen analysis was done to assess sperm quality parameters, including semen quantity, sperm density, sperm motility, and sperm morphology as recommended by the World Health Organization [14], after liquefaction at 37°C for 30 min and within 1 h of semen collection.

Semen parameters evaluated included: sperm concentration, progressive motility, total motility, and percentage of normal forms using improved Neubauer counting chamber and microscope slide examination techniques respectively [14]. The criteria for normozoospermia were defined as a concentration of 20 × 106 /ml, with sperms of forward progressive motility more than 32% of spermatozoa, total motility more than 40% and normal morphology with oval-shaped head without abnormalities of tail in at least 30% of the spermatozoa.

Measurement of metals:

Determination of lead 15 The concentration of lead in seminal plasma was determined with electrothermal atomic absorption spectrophotometer (Perkin Elmer analyst 800, Norwalk, U.S.A). Approximately 100-µl of seminal plasma was digested with 500-µl of super-grade 0.8 M HNO3 in a glass tube. The residue was dissolved in 1 ml of 1% HNO3 which was applied to a graphite tube for detection of lead. The recovery of lead in spiked semen samples was 97%. The instrument was calibrated using 0µg/L, 50µg/L, 100µg/L and 200µg/L standards for lead, respectively. A sample blank was prepared each time a set of samples to control is the assayed in order to avoid possible metal contamination from external sources. The instrument was allowed to process the sample and display the concentration in µg/L.

Measurement of mercury [16]: The concentration of mercury in seminal plasma was determined with Inductively Coupled Plasma Mass Spectrometer (Agilent 7500, Norwalk, U.S.A). The instrument was calibrated using 0ug/L, 2ug/L, 6ug/L, 10ug/L and 30ug/L standards for mercury respectively. A sample blank was prepared each set of samples to control was to be determined to avoid a possible metal contamination from external sources. Approximately 20uL of the prepared sample was aspirated into the quartz spray chamber. The instrument was allowed to process the sample and display the concentration in µg/L.

Quality control: Standard sample for each element was diluted to obtain serial dilutions of each sample and was used to calibrate and standardize the electrothermal atomic absorption spectrophotometer and Inductively Coupled Plasma Mass Spectrometer before running the analysis, and a graph was generated. Before being used all volumetric polyethylene (including the auto-sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO3 for 24 hour. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Certified reference materials (CRMs) from (Le Centre de toxicologie du, Quebec) were analyzed. 86.5ng/mL and 7.42ng/mL was obtained as lead and mercury measured level from whole blood respectively while 93.2ng/mL and 8.02 were the certified value for lead and mercury respectively.

Statistical analysis: The data obtained were statistically evaluated using statistical package for Social Science Program (SPSS) version 16.0. Values obtained in this study were represented as mean±SEM for both test and controls. Student's t test was used to compare the means while correlation was done with Pearson correlation coefficient. A P value less than 0.05 was considered statistically significant.

RESULT

Sixty (60) infertile males on routine visit to infertility clinics in Benin City, aged 38.9±0.98 formed the subject group while 20 fertile males aged 38.2±0.68 with evidence of parity were enrolled as controls.

Table 1 shows the mean concentration of lead and mercury in seminal plasma and sperm parameters in infertile males and control subjects. Lead and mercury levels were significantly higher in infertile males ($p < 0.001$) compared to control while Sperm counts, Progressive motility, and Total motility were lower ($p<0.001$) in infertile males than controls. There was however no significant different in the semen volume between infertile and fertile males.

Table 1: Seminal plasma lead, mercury levels and seminal parameters in infertile Males and Control

Measured Parameters	Infertile Fertile Subjects (N) = 60	Controls (N)=20	P-Value
Age (Years)	38.9±0.98	38.2±0.68	=0.720
Lead (ug/L)	0.99 ± 0.04	0.47 ± 0.03	<0.001
Mercury (ug/L)	0.048 ± 0.002	0.032±0.002	<0.001
Sperm Count ($x10^6$ cells/mL)	16.8 ± 2.51	74± 13.31	<0.001
ProgressiveMotility (%)	9.15 ± 1.78	57.1± 3.42	<0.001
Total Motility (%)	21.50 ± 2.36	67.05± 2.4	<0.001
Morphology (%)	15.21± 2.27	54.2± 2.57	<0.001
Semen Volume (mL)	2.98 ± 0.18	3.04 ±0.31	=0.879

Table 2 shows correlation of lead with semen parameters. Semen plasma lead correlated negatively with sperm count, progressive motility, total motility and morphology (p< 0.001) while lead correlated positively with semen volume but the correlation was not however significant (p=0.434).

Table 2: **Correlation of lead levels with Semen Parameters in infertile males**

Parameters	r- value	p- value
Sperm Count (cells/mL)	-0.440	<0.001
Progressive Motility (%)	-0.541	<0.001
Total Motility (%)	-0.527	<0.001
Morphology (%)	-0.504	<0.001
Semen Volume	0.089	=0.434

Seminal lead correlated negatively with sperm count, progressive motility, total motility and morphology while semen volume correlated positively with semen lead. The associations between lead and sperm indices were not statistically significant (table 3).

Table 3: **Correlation of lead levels with semen parameters in fertile males**

Parameters	r- Value	p- Value
Sperm Count (cells/mL)	-0.122	=0.597
Progressive Motility (%)	-0.090	=0.698
Total Motility (%)	-0.123	=0.594
Morphology (%)	-0.265	=0.247
Semen Volume	0.068	=0.770

Table 4: **Correlation of mercury levels with Semen Parameters in infertile males**

Parameters	r-value	p-value
Sperm Count (cells/mL)	-0.341	=0.002
Progressive Motility (%)	-0.382	<0.001
Total Motility (%)	-0.387	<0.001
Morphology (%)	-0.375	<0.001
Semen Volume	0.113	=0.318

Table 4 shows that seminal mercury correlated negatively with sperm count (p<0.002), progressive motility, total motility and morphology (p<0.001) while the correlation(r=0.113;p=0.318) between mercury and semen volume was not significant (p=0.318).

Table 5: Correlation of mercury levels with semen parameters in fertile males

Parameters	r- Value	p- Value
Sperm Count (cells/mL)	-0.091	=0.694
Progressive Motility (%)	0.119	=0.606
Total Motility (%)	0.043	=0.853
Morphology (%)	-0.123	=0.595
Semen Volume	0.119	=0.607

Table 5 shows no statistical significant correlation between mercury levels and sperm counts (r=-0.091;p=0.694), progressive motility (r=0.119;p=0.606), total motility (r=0.043;p=0.853), semen volume(r=0.119;p=0.607) and morphology (r=-0.123;p=0.595) in fertile males.

DISCUSSION

This study evaluated the levels of lead and mercury in seminal plasma of infertile males in Benin City, Nigeria without occupational exposure and correlated their levels with semen quality. There was significant increase (p<0.001) in seminal plasma lead and mercury concentrations in infertile males compared with controls. Statistically significant inverse correlation (p<0.001) was observed between lead and mercury levels and sperm count, progressive motility, total motility and morphology in infertile males. The increased levels of toxic metals may have contributed to low sperm counts, progressive motility, total motility and morphology in these subjects. However there was no significant different in the semen volume in infertile males compared with controls (p=0.879).There was no statistical significant association between lead and mercury levels with sperm parameters in fertile control subjects. Although not much study has been done on the effect of lead on human reproductive function in Nigeria but the few available studies elsewhere presented conflicting findings [14,17]. However we observed statistically significant increased (p<0.001)in levels of seminal plasma lead in infertile males compared with controls. This is consistent with that reported by other authors [17,18-20].The subjects evaluated in this study had no history of lead poisoning none where they occupationally exposure to lead. This is an indication that low-level exposure to environmental lead may be implicated in the decrease semen quality. It was observed that environmental lead exposure levels depending on duration may lead to disruption of both hypothalamus and pituitary glands functions in both experimental animals and humans which may result in hormonal imbalance and hence poor spermatogenesis and sperm development[21-23]. When gonadotropins interact with specific receptors on the reproductive cell surface they stimulate gonadal steroidogenesis and gametegenesis, but increased lead concentration in the tissue have been observed to interact with these receptors on the surface of the cell membrane thereby preventing the gonadotropins from binding to receptors [24] or cause increase in the production of reactive oxygen species that affect membrane integrity [25]. This interaction impairs gonadotropic binding, steroidogenesis,

and gamete growth. Lead and cadmium can induce oxidative stress by their capacity to interact with reactive oxygen species (ROS) which has both physiological and pathological role, thereby increasing their oxidant activity affecting sperm membrane integrity thus poor sperm quality [26]. Lead has also been shown to increase ROS production which disrupts the inner and outer mitochondrial membranes [27]. The increased oxidative stress may results in the release of cytochrome C protein from the mitochondria, which activates caspases, an enzyme that induces sperm cells apoptosis [27]. Xu et al[28] earlier observed a positive correlation between lead level and degradation of DNA bases in sperm cells resulting in sperm cell death, consequently abnormal semen parameters. Others also demonstrated that lead decreases sperm function such as premature acrosome loss and decreased chromatin condensation [19]. In humans, zinc may contributes to sperm chromatin stability and binds to protamine 2. Lead competes with zinc and binds human protamine 2 causing conformational changes in the protein [29]. This decreases the concentration of DNA protamine 2 binding which probably leads to alterations in sperm chromatin condensation [30]. We however observed no significant change in the semen volume of the subjects, which inferred that toxic metals may have no effect on the seminal vesicle. This observation is consistent with that reported by Saleh et al [20].

Mercury has been observed to affect male reproductive function of occupationally exposed individuals and spermatogenesis in animals [31-32]. In vitro studies have also indicated that mercury is capable of inducing sperm abnormality [33], but the role of increased seminal plasma mercury levels is not fully elucidated in occupationally unexposed individuals. In this study we observed increased seminal plasma mercury levels in infertile males compared with controls. These finding is consistent with previous studies elsewhere [34-35]. The major mechanism involved in toxic reproductive effect of mercury is oxidative stress [36]. Oxidative stress is involved in many aspect of male infertility. A shift to a more oxidative state may lead to lipid peroxidation, DNA damage, membrane alteration, making worse the metabolism and inactivation of enzymes in spermatozoa [34,37-39]. Sharma and Agarwal [39] observed that oxidative stress mediated damage to sperm membrane and may account for defective sperm function observed in high proportion of infertility cases. Also Arabi and Heydarneja [35] observed that lipid peroxidation may result in altered viability and movement which may have accounted for the abnormal morphology and motility observed in this study. In addition, membranes of acrosomal cap, the mid-piece and the tail of the human sperm have been shown to be the potential binding sites for mercury [33]. Subsequently, disruptions of sperm membrane permeability, mitochondrial function, and DNA synthesis by the microtubules are possible mechanisms whereby mercury toxicity occurs[31,331]. Apart from the sperm themselves, supporting cells in the testis and epididymis are also possible targets of mercury toxicity [33,40], which may result to semen abnormality and clinical infertility.

The major possible sources of non-occupational exposure may include environmental discharge of toxic metals containing products such as petroleum products, the use of combination of fossil fuels (petroleum and coal) as well as municipal wastes may be contributing factors to airborne lead and mercury pollution [41]. Treatment options of subjects with heavy metals toxicity is evolving even though no agreement exist for now. Clinical protocol involved the use of EDTA, DMPS and DMSA chelators which had proved to be therapeutically beneficial [42].

Limitation of study: The inability to obtain large sample size due to the reluctance of prospective patients and control subjects to give consent for their samples to be collected and used was a major limitation in this study.

CONCLUSION

In conclusion, based on the result of this study lead and mercury may represent reproductive toxicant in occupationally unexposed infertile males in Benin City, Nigeria. Toxic metals may be routinely assayed when evaluating male subjects with idiopathic infertility.

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Conflict of Interest : None

REFERENCES

1. Hovatta O, Vena la inen E, Kuusima ki L, Heikkila J, Hirvi T, Reima I. Aluminium, lead and cadmium concentrations in seminal plasma and spermatozoa and semen quality in Finnish men. Hum Reprod 1998;13(1):115-119.
2. Emokpae MA,Uadia PO, Omale- Itodo A, Orok TN. Male infertility and Endocrinopathies in Kano, North Western Nigeria. Annals Afr Med 2007; 6(2): 64 – 67.
3. Sharpe RM, Skakkebaek NE. Are oestrogens invoved in falling sperm counts and disorders of the male genital tract? Lancet 1993;341:1392-1395.
4. Skakkebaek NE, Keiding N. Changes in semen and the testis. Br Med J 1994; 309:1316-1317.
5. Chia SE, Ong CN, Lee ST et al. Blood concentrations of lead, mercury, zinc and copper and human semen parameters. Arch Androl 1992;29:177-183.
6. Hu WY, Wu SH, Wang LL et al. A toxicological and epidemiological study on reproductive functions of male workers exposed to lead. J Hyg Epidemiol Microbiol Immunol 1992; 36:25-30.

7. Xu B, Chia SE, Tsakok M et al. Trace elements in blood and seminal plasma and their relationship to sperm quality. Arch Androl 1993; 29:177-183.
8. Alabi NS, Whanger PD, Wu AS. Interactive effect of organic and inorganic selenium with cadmium and mercury on spermatozoa oxygen consumption and motility in vitro. Biol Reprod 1985;33:911-919.
9. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D et al. Cadmium, lead and other metals in relation to semen quality: Human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect 2008;116: 1473-1479.
10. Wyrobek AJ, Schrader SM, Perreault SD, Fenster L, Huszar G, Katz DF Et al. Assessment of reproductive disordersand birth defects in communities near hazardous chemical sites. ReprodToxicol 1997;11: 243-259.
11. Apostoli P, Kiss P, Porru S, Bonde JP, Vanhoorne M. Male reproductivetoxicity of lead in animals and humans. ASCLEPIOS Study Group. Occup. Environ. Med 1998;55: 364–374.
12. Plechaty MM, Noll B, Sunderman FW. Lead concentrations in semen of healthy men without occupational exposure to lead. Ann Clin Lab Sci 1977;7: 515–518.
13. Lwange S, Lemeshow S. Sample size determination in health studies: A practical manual, Geneva, World Health Organization, 1991,p.23-41.
14. World Health Organization. Laboratory manual for the Examination and processing of human semen. 5th ed. WHO Press, Switzerland,2010.
15. Olmedo P, Pla A, Hernandez AF, Lopez-Guarnido O, Rodrigo L, Gil F. Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. Anal Chim Acta,2010; 65:60-67.
16. Fong BMW, Siu ST, Lee SK, Tam S. Determination of mercury in whole blood and urine by inductively coupled plasma Mas spectrometry. J Anal Toxicol 2007;31:281-287.
17. Hernández-Ochoa I, García-Vargas G, López-Carrillo L, Rubio-Andrade M, Morán-Martínez J, Cebrián ME, Quintanilla-Vega B. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. Reprod Toxicol 2005; 20: 221-228.
18. Mandiola J, Moreno JM, Roca M, Vergara-Juarez N, Martinez-Garcia MJ, Garcia-Sanchez A. (2011). Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: a pilot study. Environ Health 2011;10(6): 1-7.
19. Awadalla NJ, El-Helaly M, Gouida M, Mandour R, Mansour M. (2011). Sperm Chromatin Structure, Semen Quality and Lead in Blood and Seminal Fluid of Infertile Men. Int. J. of Occup. And Environ Med 2011;2(1): 27-36.
20. Saleh MA, Taha EA, Ismail SA, Gaber HD, Morsi HA, Ghandour NM. Lead and Cadmium assay in Serum and Semen of Infertile Men attending Andrology clinic in Assiut University Hospital (Rural versus Urban). J Environ Sci, Toxicol Food Tech 2013;2(4): 38-45.
21. Sokol RZ, Madding CE, Swerdloff RS. Lead toxicity and the hypothalamic-pituitary-testicular axis. Biol Reprod 1985; 33: 722-728.
22. McGregor AJ,Mason HJ. Chronic occupational lead exposure and testicular endocrine function. Hum ExpToxicol 1990;9: 371-376.
23. Sokol RZ, Wang S, Wan YJ, Stanczyk FZ, Gentzschein E, Chapin RE. Long-term, low-dose lead exposure alters the gonadotropin-releasing hormone system in the male rat. Environ Health Perspect 2002; 110: 871-874.
24. Benoff S, Jacob A, Hurley IR. Male infertility and environmental exposure to lead and cadmium. Hum. Reprod. Update2000; 6(2): 107–121.
25. De Rosa M, Zarrilli S.Traffic pollutants affect fertility in men. Hum Reprod 2003; 18 (5): 1055-1061.
26. Oteiza PI, Mackenzie GG, Verstraeten SV. Metals in neurodegeneration: involvement of oxidants and oxidant-sensitive transcription factors. Mol Aspects Med 2004;25 (2): 103–115.
27. Wang X, Sharma RK, Sikka SC. Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. Fertil. Steril 2003;80(3): 531-535.
28. Xu DX, Shen HM, Zhu QX, Chua L, Wang QN, Chia SE. The associations among semen quality, oxidative DNA damage in human seminal plasma. Mutat Res 2003; 534: 155-163.
29. Quintanilla-Vega B, Hoover DJ, Bal W. (2000).Lead interaction with human protamine (HP2) as a mechanism of male reproductive toxicity. Chem Res Toxicol 2000; 13: 594-600.
30. Bonde JP,Joffe M, Apostoli P. Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. Occup Environ Med 2002; 59:234-42.
31. Alaee S, Talaiekhozani A, Rezaei S, Alaee K, Yousefian E. Cadmium and male infertility. J Infertil Reprod Biol 2014;2(2):62-69.
32. Homma-Takeda S, Kugenuma Y, Iwamuro T, Kumagai Y, Shimojo N. Impairment of spermatogenesis in rats by methylmercury: involvement of stage and cell-specific germ cell apoptosis. Toxicology 2001; 169(1); 25-35.
33. Ernst E, Lauritsen JG. Effect of organic and inorganic mercury onhuman sperm motility. Pharmacol Toxicol 1991; 68(6): 440-444.
34. Arabi M. The role of mercury in etiology of sperm dysfunction in Holstein bull. Asian -Aust J Anim Sci 2006; 19: 335-340.
35. Arabi M, Heydanejd MS. In vitro mercury exposure on spermatozoa from normospermic individuals. Pak J Biol Sci 2007; 10(5): 2448-2452.
36. O'flahery CN, Beorlegui N, Beconi MT. Participation of superoxide anion in the capacitation of cryopreserved bovine sperm. Int J Androl 2003; 26: 109-114.

37. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003;79(4): 829-843.
38. Moustafa MH, Sharma RK, Thornton J, Mascha E, Abdel-Hafez MA, Thomas AJ, Agarwal A. Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Hum Reprod 2004; 19: 129-138.
39. Sharma RK, Agarwal A. Role of reactive oxygen species in Male infertility. Urology 1996;48: 835-850.
40. Working PK, Bus JS, Hamm TE. Reproductive effects of inhaledmethyl chloride in the male Fischer 344 rat: Mating performanceand dominant lethal assay. Toxicol Appl Pharmacol 1985; 77(1): 133-143.
41. Emokpae MA, Adobor CA. Association of seminal plasma cadmium levels with semen quality in non-occupationally exposed infertile Nigerian males. J Environ Occup Sci 2014; 3(4):01-04.
42. Bernhoft RA. Cadmium Toxicity and Treatment. Scientific World J 2013;2013:1-7.