JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

PRELIMINARY COMMUNICATIONS | DOI: 10.5584/jiomics.v8i1.216

Assessment of occupational exposures to multiple metals with urinary porphyrin profiles

V Lopes de Andrade [1], ML Mateus [1], M Aschner [2], AP Marreilha dos Santos [1]*

[1] Instituto de Investigação do Medicamento, iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal; [2] Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Received: 11 November 2017 **Accepted:** 5 March 2018 **Available Online**: 16 April 2018

ABSTRACT

Chronic occupational exposure to low levels of metal mixtures necessitates biomonitoring of exposed workers. However, a single biomarker is rarely sufficient to ascertain the exposure of an individual to a complex mixture, with multiparameter analysis of the same sample considered recently as a preferred approach. Porphyrins are formed as intermediates of heme biosynthesis and different metals can exert their effects at different points of this metabolic pathway, leading to changed urinary porphyrins excretion profiles. The aim of this work was to develop a model that could serve to identify, on an individual basis, multiple metal exposure resulting from mining work, by using urinary porphyrin profiles. Urine samples of workers were obtained from a Portuguese mining company and a non-occupationally exposed group was used as control. The levels of uro-, hepta-, hexa-, penta-, copro- and protoporphyrins were determined by HPLC. It was observed that only heptaporphyrin levels in miners were significantly ($p<0.05$) different from controls. However, when the concentrations of all porphyrins were combined by binary logistic regression, their ability to discriminate between miners and controls was higher than each one of the porphyrins alone, as indicated by a greater curve area under a Receiver Operating Characteristics (ROC) curve. Moreover, when the combined porphyrins were used to calculate the probability of each subject fit in the occupationally exposed group, 83% of 47 individuals were correctly identified with respect to their type of exposure. These results suggest that the integration of the urinary porphyrin profile is a promising tool for the detection of subjects exhibiting biochemical modifications due to occupational exposure to metals in mines.

Keywords: metal mixture, occupational exposure, urine porphyrin profiles, biomarkers

Abbreviations: BM:biomarkers; HPLC: High Performance Liquid Chromatography; ROC: Receiver Operating Characteristics; uro-: uroporphyrins; hepta-: heptaporphyrins; hexa-: hexaporphyrins; penta-: pentaporphyrins; copro-: coproporphyrins; proto-: protoporphyrins; *oem*: occupationally exposed in a mine

1. Introduction

Mining represents one of the most hazardous occupations [1], and has been long recognized as arduous and liable to injury and disease [2]. Biomonitoring of chronic occupational exposure to low levels of metal mixtures in miners as well as other occupations is indispensable for workers' health, with molecular biomarkers (BM) representing critical tools in achieving this task [3, 4]. However a single BM is rarely sufficient to ascertain exposure in a single person to a complex mixture. Accordingly, information from multivariate analysis of multiple parameters, measured simultaneously in the same sample is being considered as a promising approach [5]. Porphyrin analysis represents one such approach. Porphyrins are compounds formed as intermediates of heme biosynthesis [6], and it well established that various metals can exert their effects at different points of the porphyrin metabolic pathway, leading to changed urinary porphyrin excretion profiles. Thus, porphyrins have been recognized as promising BMs of metal mixture-induced toxicity [7]. Indeed, previous work in our laboratory revealed that changes in rat urinary porphyrin profiles could predict the magnitude of effects induced by a metal mixture in individual animals [8, 9]. Our recent work has focused on the translation of these

***Corresponding author:** AP Marreilha dos Santos . Tel: +351 217946400 ; E-Mail Adress: apsantos@ff.ulisboa.pt.

observations to human populations exposed to metal mixtures, such as mine workers [3]. The aim of this study was to develop a model that could serve to identify multiple metal exposures resulting from mining work taking advantage of urinary porphyrin profile analysis on an individual basis.

2. Material and Methods

Urine samples from workers in a mining company in Portugal were obtained from the Occupational Health Department under the supervision of a nurse and doctor; The working group (n=29) was composed of male miners aged between 24 and 56 years. All of them were working in the mine for more than one year, A non-occupationally metal exposed group composed of workers from the same company (also male individuals aged between 22 and 60) was used as a control group. Individuals in the control group were not exposed to metals in their work environment (n=20). All the biological samples were collected on the last day of the week and stored at –80°C until the analysis.

Chromatographic porphyrin analysis was performed by HPLC after sample preparation according to Woods et al, 2009 [8] to obtain porphyrin profiles by determining uro-, hepta-, hexa-, penta-, copro- and protoporphyrins concentrations.

Statistical analysis was performed with the SPSS 16.0 statistical package for Windows (SPSS, Inc., Chicago, IL, USA). Data are expressed as means ± standard deviations (SD). 1) The occupational and non-occupational exposed groups were compared by Mann-Whitney U tests, respecting to their levels of each porphyrin. 2) The ability of urinary porphyrins to detect occupational exposures in mines was evaluated by ROC analysis, which is a statistical tool that can be used to evaluate the diagnostic accuracy of BMs, alone or in combination [10]. In this analysis, BMs levels are plotted in a curve under a 1- Specificity (x) vs Sensitivity (y) axis, being the area under the curve directly proportional to the BM(s) diagnostic accuracy. Actually, these plots of 1-Specifity (false positives or 1-true negatives) versus Sensitivity (true positives) is an effective measure of BM(s) accuracy [11]. Thus, an area=100% indicates that all subjects are true positives and true negatives revealing a maximum accuracy of the BM(s); by its turn an area that lies close or under 50% have no information content and indicate that the BM(s) do not have diagnostic utility (Warnock and Peck, 2010)[12]. Here, the levels of each porphyrin alone and the combination all the porphyrins by binary logistic regression, were tested by ROC analysis; the obtained areas were compared to evaluate which BM(s) exhibited the highest diagnostic accuracy. 3) Posteriorly we created a mathematical expression that could serve to determine the odds of a new subject under study being exposed to metals in his work environment [13]; The odd values was calculated using levels of urinary porphyrins. This expression was generated by binary logistic regression. Notably, combining porphyrins profiles had a significantly better diagnostic accuracy under the ROC curves for metal exposure.

3. Results

3.1 Urinary porphyrin profiles

Figure 1 shows that a significant ($p<0.05$) difference and lower urinary levels of heptaporphyrins were noted in miners when compared to controls.

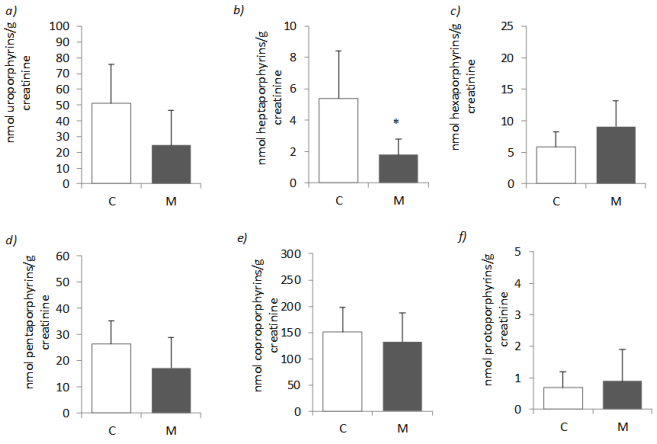


Figure 1. Uroporphyrin (a), heptaporphyrin (b), hexaporphyrin (c), pentaporphyrin (d), coproporphyrin (e) and protoporphyrin (f) urinary levels in controls (C) and miner (M) population. Data represent the mean ± SD and n=20 and 29 in C and M groups, respectively. All groups were compared by Mann-Whitney U tests. * represents $p < 0.05$ versus C.

3.2 Capability of urinary porphyrins to detect occupational exposures in mines

The diagnostic accuracy of each porphyrin alone was tested for their ability to detect subjects occupationally exposed in mines (true positives). This approach was deemed unsatisfactory as the areas under the 1- Specificity (x) vs Sensitivity (y) axis were lower than 50%, attesting to high false positives and false negatives, and indicating lack of diagnostic utility of each of the BMs alone (Figure 2a). Alternatively, when all the porphyrins were combined by binary logistic regression, the number of true positives and true negatives increased substantially as reflected by the increase in the area under the ROC curve (area= 0.743; $p<0.05$) (Figure 2b).

3.3 Detection of mining work related exposure in an individual basis

To detect the mining work related exposure in each subject, we developed a mathematical expression. The expression below represents the odds of a subject to be considered as exposed in a mine (oem), by depicting the concentrations of their urinary porphyrins (uro-, hepta-,

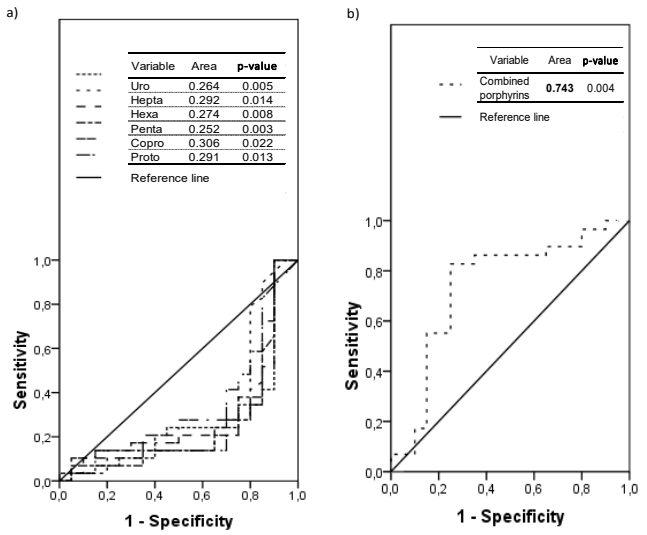


Figure 2. The ability of the urinary levels of uroporphyrins, hepta-porphyrins, hexaporphyrins, pentaporphyrins, coproporphyrins and protoporphyrins individually (a) and their combination by binary logistic regression (b) to identify subjects with mining related exposures. The curve's area under the 1- Specificity (x) vs Sensitivity (y) axis are plotted by ROC analysis: areas are directly proportional to the BMs discriminant capabilities. All represented areas reached statistical significance ($p<0.05$). N=49.

hexa-, penta-, copro- and protoporphyrins), by binary logistic regression:

$$ODDs\ (oem) = 26.6Uro - 176.3Hepta + 484.9Hexa$$
$$+111.3Penta + 5.9Copro + 790.2Proto + 1.4$$

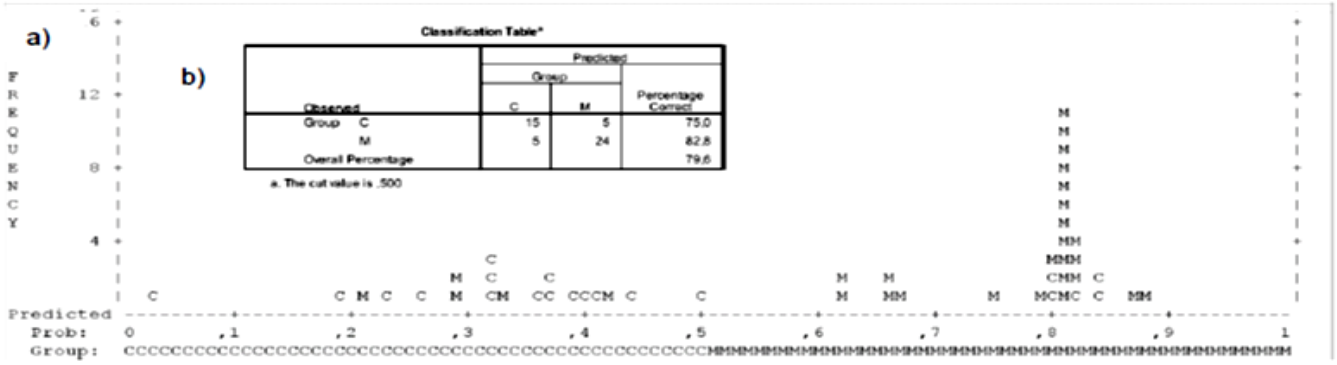
The use of combined urinary porphyrin levels leads to 83% of correct detections of occupational exposure in miners (Figure 3b). More precisely, 15 of 20 subjects included in the control group were correctly identified as non-occupational exposed exhibiting only a 0.15 to 0.45 range odd of being a miner (Figure 3b). Additionally, 4 of the 5 controls were erroneously classified as miners showing a 0.80 to 0.85 range odd of being a miner, while one person had an odd of only 0.5 (Figure 3a). With regard to the miners, 24 of the 29 workers were correctly identified as miners with odds that ranged from 0.6 to 0.9 with a higher frequency at the odd of 0.8 (Figure 3a). Concerning to the false negatives, 5 miners were incorrectly included in the control group and a 0.20 to 0.45 range odd of being a miner was attributed to these persons (Figure 3a).

4. Discussion

Low chronic exposure to metals may result in insidious poisoning that may manifest clinically at a very late stage. Thus it is critical to monitor mine workers so that subclinical toxicity can be identified at the onset of disease or even at earlier stages [3, 14]. While molecular BMs represent key tools in occupational medicine [15], urinary porphyrin profiles may serve as BMs of exposure to toxic metals in humans [16, 17]. Since various metals can exert their effects at different points of the heme synthesis pathway, we hypothesized that the urinary porphyrin profile might serve as a "fingerprint" that would permit a satisfactory identification of individuals exposed to metals due to their occupation in mines.

Our results showed that each of the porphyrins alone poorly detected occupational exposures to metals, even on a group basis, with miners and controls exhibiting differences that lacked statistical significance ($p>0.05$) (Figure1). Miners are exposed to several metals [3], such as lead (Pb), arsenic (As), manganese (Mn), mercury (Hg) and aluminum (Al), which interfere at common, and also at different points, in the heme synthesis sequential pathway [18, 19, 20, 21, 22]. These events may lead to distinct fingerprint in urinary excretion of each of the porphyrins, explaining their poor diagnostic performance when used alone. These results were confirmed by the observation of areas <0.5 under a 1-Specificity (x) vs Sensitivity (y) axis plotted by ROC analysis (Figure 2a). Indeed, BMs with a curve that lies close or under the diagonal reference line (area = 0.5) had no information content, and therefore no diagnostic utility [12]. In contrast,

Figure 3. Predicted probability of each subject belong to the mining group (M) after application of Expression 1 to each studied individual. Each symbol (C or M) represents 1 subject (a) and the ODDs cut value for miners group membership is ≥ 0.5. The table (b) represents the counts of subjects included in each group as predicted by the model vs the real group (observed). N=20 and 29 in C and M groups, respectively.



when the urinary porphyrins profile was applied as a whole with integration of all the porphyrins by binary logistic regression, the area under a ROC curve increased the diagnostic utility (area=0.743) (Figure 2b) [12]. Therefore, the concentrations of all the porphyrins were used to generate an equation to determine the odds of a new subject under analysis to be occupationally exposed in a mine (previously displayed expression). When this equation was tested in the studied subjects, a correct detection of the occupational exposure in the mines was obtained for 83% of the cases (Figure 3b). We suggest that the called "erroneous classification" is not directly related to the model, but rather with the type of environmental exposure of all the populations; the control as well as the mining populations are not homogeneous in terms of work, housing location, social habits and genetic susceptibility. Our results are satisfactory for a preliminary study with N=49, obtaining 25% of false positives and 17% of false negatives. These findings also lead us to proceed with additional future work, expanding on the sample size, to further establish the proof-of-concept of these analyses as predicitive tools of metal mixture exposures.

5. Concluding Remarks

Overall, the integration of the urinary porphyrin profile showed superiority in discriminating miners or subjects non -occupationally exposed, than any of the porphyrin concentrations alone. The proposed multiparameter approach is promising for the detection of mining work related to multiple metal exposures, and should be further validated in future studies. Although our study was focused on porphyrin profile and metals, we may add that metals as well as metal mixtures may increase oxidative stress and originate adverse effects including the neurotoxic ones [23]. In fact, Pb, Hg, Fe and Mn are typical examples of metal transport and toxicity at barriers, like the blood brain barrier, inducing neurotoxicity [24]. Moreover, toxic metals can promote the disruption of essential metal homeostasis, and minor alteration in quantity, form or place of these vital elements may lead to essential metal imbalances, associated with several diseases [25].

Our studies highlight the need for additional time course analyses to better understand gene-omics relationships and adverse outcome pathways. This is important if transcriptomics, proteomics, metabolomics and other omics approaches are to be used together to investigate neurotoxicity or as bio-monitoring tools for exposure.

Acknowledgments

Financial support from FCT strategic project PEst-OE/SAU/UI4013/2011, Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa and from the National Institute of Health (NIH R01ES10563, R01ES07331)

References

1] P. Coelho, S. Costa, S. Silva, A. Walter, J. Ranville, A.C.A. Sousa, C. Costa, M. Coelho, J. García-Lestón, M. Ramiro Pastorinho, B., Laffon, E. Pásaro, C. Harrington, A. Taylor, J.P. Teixeira, J Toxicol Environ Health 75 (2012) 893-908. DOI: 10.1080/15287394.2012.690705

2] A.M. Donoghue, Occup Med. 54 (2004) 283-289. DOI: 10.1093/occmed/kqh072

3] S.V. Dhatrak, S.S. Nandi, Indian J Occup Environ Med 13 (2009) 60-64. DOI: 10.4103/0019-5278.55121

4] P.A. Schulte, J.E. Hauser, Toxicol Lett 213 (2011) 91-99. DOI: 10.1016/j.toxlet.2011.03.027

5] J.M. Christensen, Sci Total Environ 166 (1995) 89-135. DOI: 10.1016/0048-9697(95)04478-J

6] B. Quintanilla-Vega, A. Hernandez, T. Mendoza-Figueroa, Toxicol in Vitro 10 (1996) 675-683. PMID: 20650251.

7] G. Wang, B.A. Fowler, Toxicol Appl Pharmacol 233 (2008) 92–99. DOI: 10.1016/j.taap.2008.01.017

8] J.S. Woods, M.D. Martin, B.G. Leroux, T.A. DeRouen, M.F. Bernardo, H.S. Luis, J.G. Leitão, P. L. Simmonds, T.C. Rue, Clin Chim Acta 405 (2009) 104-109. DOI: 10.1016/j.cca.2009.04.014

9] V. Andrade, M.L. Mateus, M.C. Batoréu, M. Aschner, A.P. Marreilha dos Santos, Neurotoxicology 45 (2014) 168-177. DOI: 10.1016/j.neuro.2014.10.009

10] D. Liu, X.-H. Zhou, Acad Radiol 20 (2013) 874–882. DOI: 10.1016/j.acra.2013.03.009

11] K. Hajian-Tilaki, Caspian J Intern Med 4 (2013): 627–635. PMCID: PMC3755824.

12] D. Warnock, C. Peck, Nature Biotechnology 28 (2010) 444–445. DOI: 10.1038/nbt0510-444

13] R. Brant, Biometrics 46 (1990) 1171-1178. DOI: 10.2307/2532457

14] P. Kakkar, F.N. Jaffery, Environ Toxicol Pharmacol 19 (2005) 335-349. DOI: 10.1016/j.etap.2004.09.003

15] G. Scherer, Exp Toxicol Pathol 57 (2005) 75–110. DOI: 10.1016/j.etp.2005.05.007

16] J. Li, Z. Cai, S. Xu, C. Liao, X. Song, L. Chen, J Liq Chromatogr R T 34 (2011) 1578-1593. DOI: 10.1080/10826076.2011.575981

17] J.C. Ng, J.P. Wang, B. Zheng, C. Zhai, R. Maddalena, F. Liu, Toxicol Appl Pharmacol 206 (2005) 176–184. DOI: 10.1016/j.taap.2004.09.021

18] M. Ahamed, M.K.J. Siddiqui, Clin Chim Acta 383 (2007) 57-64. DOI: 10.1016/j.cca.2007.04.024

19] K.M. Kadish, K.M., Smith, R. Guilard (Ed.) The Porphyrin Handbook: Medical aspects of porphyrins, Volume 14, Academic Press, Elsevier Science, 2012

20] M. Krishnamohan, L. Qi, P.K.S. Lam, M.R. Moore, J.C. Ng, Toxicol Appl Pharmacol 224 (2007) 89–97. DOI: 10.1016/j.taap.2007.04.020

21] M.D. Maines, Biochem J 190 (1980) 315-321. DOI: 10.1042/bj1900315

22] J.S. Woods, M.D. Martin, C.A. Naleway, D. Echeverria, J Toxicol Environ Health 40 (1993) 235-246. DOI: 10.1080/15287399309531791

23] M. Farina, D.S. Avila, J.B. da Rocha, M. Aschner, Neurochem Int 62 (2013) 575-94. DOI: 10.1016/j.neuint.2012.12.006

24] W. Zhang, M. Aschner, J.F. Ghersi-Egea, Toxicol Appl Pharmacol 192 (2003) 1-11. PMID: 14554098

25] S. DoKer, S. Manicou, M. Dogan, R. Lobinski, Metallomics 2 (2010) 549-555. DOI: 10.1039/c004508j