SHORT REPORT

Causes of death among lead smelters in relation to the glucose-6-phosphate dehydrogenase polymorphism

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Objective: To assess, by updating a follow-up mortality study of a lead smelters cohort in Sardinia, Italy, the adverse health effects following occupational lead exposure in relation to the glucose-6-phosphate dehydrogenase (G6PD) polymorphism.
Method: The 1973–2003 mortality of 1017 male lead smelters were followed-up, divided into two subcohorts according to the G6PD phenotype: whether G6PD deficient (G6PD−) or wild-type (wtG6PD). Deaths observed in the overall cohort and the two subcohorts were compared with those expected, on the basis of the age-, sex- and calendar year-specific mortality in the general male population of the island. Directly standardised mortality rates (sr) in the two subcohorts were also compared.
Results: Cardiovascular mortality was strongly reduced among production and maintenance workers, which is most related to the healthy worker effect. However, the sr for cardiovascular diseases was substantially lower among the G6PD− subcohort (5.0×10^{-4}) than among the wtG6PD subcohort (33.6×10^{-4}; $\chi^2=1.10$; p=NS). Neoplasms of the haemopoietic system exceeded the expectation in the G6PD− subcohort (SMR=388; 95% CI 111 to 1108). No other cancer sites showed any excess in the overall cohort or in the two subcohorts. No death from haemolytic anaemia occurred in the G6PD− subcohort.
Conclusion: With due consideration of the limited statistical power of our study, previous results suggesting that in workplaces where exposure is under careful control, expressing the G6PD− phenotype does not convey increased susceptibility to lead toxicity are confirmed. The observed excess risk of haematopoietic malignancies seems to have most likely resulted from chance.

A preliminary study of a lead smelters cohort showed that overall mortality was not increased among subjects expressing the glucose-6-phosphate dehydrogenase (G6PD)-deficient phenotype (G6PD−) compared with their wild type G6PD (wtG6PD) work mates,[1] not differing by average blood lead level and environmental lead exposure.[1 2] Although statistically irrelevant, such results were the first published that contrasted with anedoctal reports of a greater sensitivity of G6PD− subjects towards lead haemotoxicity. The European regulation excluding these subjects from jobs conveying possible lead exposure was successfully challenged as posing a discrimination on a genetic base without any scientific support for a preventive effect.[3] Besides, the mortality follow-up of a cohort of G6PD− subjects identified in a population screening provided preliminary evidence of a strong reduction in mortality from cardiovascular diseases, and particularly ischaemic heart disease,[4] supporting early results from a US cross-sectional study.[5]

G6PD is a polymorphic enzyme providing most of the reduced form of extramitochondrial nicotinamide-adenine-dinucleotide phosphate by ruling the metabolic step from glucose-6-phosphate to 6-phosphogluconolactone in the pentose phosphate pathway.[6] Nicotinamide-adenine-dinucleotide phosphate intervenes as a redox donor in numerous enzymatic reactions, including regeneration of the reduced form of glutathione, a major defence against oxidative damage, particularly in erythrocytes, which may undergo haemolysis following ingestion of fava beans or several drugs in subjects with the G6PD-deficient phenotype.[7] The G6PD gene is located in the Xq28 chromosome, and therefore hemizygous men are more frequently affected by polymorphisms associated with reduced or null enzyme activity.[6 7] Due to the high prevalence of G6PD gene variants associated with extremely low enzyme activity (0–7%),[6] the Italian region of Sardinia is an ideal setting for studies of health effects related to the G6PD polymorphism. Aiming to investigate risk for specific groups of causes of death, we extended the follow-up of the lead smelters cohort up to 31 December 2003.

MATERIALS AND METHODS

The characteristics of the cohort were previously described in detail.[1] Briefly, it consisted of 1384 subjects employed at any time in a lead smelting plant located in south-western Sardinia, Italy, from 1 January 1973, when the plant officially started operating, up to 31 December 1990. After excluding female employees (n = 18) and unidentified subjects (n = 26), the cohort comprised of 323 (24.1%) administrative employees and production supervisors, and 1017 (74.4%) production and maintenance workers. Among the latter, very few (n = 10; 1.0%) ever changed their job over the period of enrolling in the cohort. Information on the G6PD phenotype was retrieved and abstracted from the industry medical records for 933 (91.7%) production and maintenance workers. The G6PD phenotype was assessed during the routine medical screening at hiring with a modified Beutler's fluorescent spot test.[8]

The present study was limited to the 933 production and maintenance male workers with known G6PD phenotype, divided into two subcohorts according to the G6PD phenotype: 748 wtG6PD and 185 G6PD−. The vital status of cohort members was searched up to 31 December 2003 at the population registrar of the last known municipality of residence. For these subjects, follow-up was 99.5% successful, with only five subjects (four expressing the wtG6PD and one the G6PD− phenotype) lost, contributing to follow-up through the last day of known vital status. The cause of death of all the 102 deceased cohort members was identified at the Registrar of the Causes of Death of the Public Health Department of the local health units where death occurred, and coded according to the International Classification of Diseases—9th revision.

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; G6PD−, glucose-6-phosphate dehydrogenase deficient; SMR, standardised mortality ratio; sr, standardised mortality rate; wtG6PD, wild-type glucose-6-phosphate dehydrogenase

Observed deaths were compared with those expected for each cause of interest, based on the 5-year age group-specific and calendar year-specific mortality in the regional male population. The standardised mortality ratio (SMR) and its 95% CI were then calculated, as 100 times the observed/expected ratio. Directly standardised mortality rates (sr) in the two subcohorts were also calculated, with the 1981 census regional male population as the standard. The 95% CI of the mortality rates was based on the normal approximation to the binomial distribution.

The ethics committee of the University Polyclinic, Cagliari, Italy approved the study protocol.

RESULTS

The mean (SD) age at entering follow-up was similar between the two subcohorts (wtG6PD: 31.6 (8.43) years; G6PD−: 31.0 (8.62) years), and also the duration of employment was as of 31 December 1991, when the first follow-up study ended (wtG6PD: 11.5 (6.33) years; G6PD−: 11.1 (6.05) years). Table 1 shows the cause-specific SMRs by subcohort of G6PD phenotype and in the overall cohort.

Overall mortality was significantly decreased (SMR = 56; 95% CI 46 to 68), mainly due to a 63% reduction in cardiovascular deaths (SMR = 37; 95% CI 25 to 65). The healthy worker effect, resulting from medical selection at hiring, is a most plausible explanation for such finding. However, the decrease in cardiovascular deaths was particularly strong among the G6PD− subcohort, with only three observed deaths versus 14.2 expected (SMR = 21; 95% CI 7 to 59). Only one death from haemolytic anaemia occurred in the cohort versus 0.7 expected. This was a case of deadly acute arsine poisoning in a wtG6PD subject, leading to an acute haemolytic crisis and acute renal failure. No deaths occurred from anaemia among the G6PD− subcohort versus 0.1 expected. Mortality from cancer was close to that expected in both subcohorts and in the total cohort. Among specific cancer sites, an almost fourfold significant increase in the SMR was observed in the G6PD− subcohort for cancer of the haemopoietic system, based on three deaths (SMR = 388; 95% CI 111 to 1108). Compared with the wtG6PD subcohort (sr 0.9×10^{-4}; 95% CI 0.0 to 2.1), the sr from haemopoietic system malignancies among the G6PD− subcohort (sr 20.7×10^{-4}; 95% CI 7.2 to 34.2) was significantly elevated ($\chi^2 = 4.92$; $p<0.05$). The sr for cardiovascular mortality was substantially decreased in the G6PD− subcohort (G6PD−: sr 5.0×10^{-4}; 95% CI 0.0 to 11.6; wtG6PD: sr 33.6×10^{-4}; 95% CI 25.7 to 41.3; $\chi^2 = 1.10$; p = NS). No other malignant or non-malignant causes of death showed a significant difference between the two subcohorts.

DISCUSSION

A preliminary mortality study of this cohort suggested that subjects expressing the G6PD− phenotype had not experienced an increase in total mortality, cancer mortality and cardiovascular mortality compared with the wtG6PD coworkers after occupational exposure to lead. In particular, no death from anaemia was observed among the G6PD− subcohort.[1] However, the period of follow-up was too short, the average age of cohort members too young, and the size of the G6PD− subcohort insufficient to exclude an effect in relation to the G6PD− phenotype. Extending follow-up for an additional 12 years allowed to accumulate a sizeable number of deaths, and to explore mortality in some more detail. Confirming previous reports,[4 5] we observed a substantial decrease in cardiovascular mortality and a significant increase in mortality from haematologic malignancies among the G6PD− subcohort. However, the statistical power was still insufficient to infer protection from the genetic condition against cardiovascular death, and a recent case–control study of lymphoma has not shown an association with the GdMed+ genotype, the most common allele associated with deficient enzyme activity in the Sardinian population (Cocco *et al*[9]).

The small size of the cohort and the low overall mortality resulting from pre-employment selection still do not allow conclusive inference. Nonetheless, despite the increased prevalence of the genetic trait in the study area, the present

Table 1 Standardised mortality ratio for specific causes by glucose-6-phosphate dehydrogenase phenotype subcohorts, and in the overall cohort of production and maintenance workers in a lead smelting plant

	wtG6PD subcohort (Py = 2615.58)			G6PD− subcohort (Py = 5324.87)			Total cohort (Py = 26 940.45)		
	Obs	Exp	SMR (95% CI)	Obs	Exp	SMR (95% CI)	Obs	Exp	SMR (95% CI)
All causes (0.0–999.9)	81	143.2	57 (46 to 70)	21	40.0	53 (35 to 81)	102	182.8	56 (46 to 68)
All cancers (140.0–208.9)	36	37.9	95 (69 to 132)	8	9.6	83 (42 to 167)	44	47.5	93 (69 to 125)
Cancer of the digestive system (150.0–159.9)	11	12.4	89 (49 to 160)	2	3.2	63 (16 to 248)	13	15.6	83 (48 to 143)
Cancer of the respiratory system (160.0–165.9)	16	13.0	123 (76 to 201)	1	3.1	32 (5 to 206)	17	16.1	106 (66 to 170)
Cancer of the urinary system (185.0–189.9)	2	4.0	50 (13 to 193)	2	1.2	174 (44 to 683)	4	5.2	77 (29 to 205)
Cancer of the haemopoietic system (200.0–208.9)	1	3.1	33 (5 to 210)	3	0.8	388 (111 to 1108)	4	3.8	104 (39 to 278)
Anaemia (280.0–285.9)	1	0.6	178 (26 to 1231)	0			1	0.7	142 (20 to 1000)
Cardiovascular diseases (390.0–459.9)	20	47.5	42 (28 to 64)	3	14.2	21 (7 to 59)	23	61.7	37 (25 to 55)
Respiratory diseases (460.0–519.9)	6	9.9	60 (27 to 134)	4	3.0	134 (51 to 357)	10	12.9	78 (42 to 144)
Digestive system diseases (520.0–579.9)	5	11.8	42 (18 to 99)	2	3.0	67 (17 to 2655)	7	14.8	47 (23 to 97)

EXP, expected; G6PD, glucose-6-phosphate dehydrogenase; OBS, observed; Py, person years; SMR, standardised mortality ratio; wtG6PD, wild-type glucose-6-phosphate dehydrogenase.

study population is unique in that, to the best of our knowledge, no other industrial cohorts in the study area or elsewhere in the world had the G6PD phenotype during pre-employment tests. The decision of including the G6PD polymorphism in the pre-employment screening programme was not meant to assess lead susceptibility in the individual workers. Instead, it resulted from the vast concern among local health professionals to reduce the seasonal occurrence of haemolytic crises following ingestion of raw or cooked fresh fava beans (favism), the only disorder known to be associated to the G6PD− phenotype. In fact, since the early 1970s, screening programmes have been promoted by the regional health department, including routine screening of the G6PD polymorphisms in all newborns.

In conclusion, the update of the lead smelters cohort study in Sardinia, Italy, confirms that, once workplace exposure to lead is kept under regular control, subjects expressing the G6PD− phenotype do not manifest an increased susceptibility to adverse health outcomes with respect to their wtG6PD work mates. Additional studies are warranted to exclude an increase in the risk of haemolytic anemia, and to explore in more detail the risk for specific disease entities.

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