

## Lead Poisoning Induced Severe Hemolytic Anemia, Basophilic Stippling, Mimicking Erythrocyte Pyrimidine 5'-nucleotidase Deficiency in Beta Thalassemia Minor

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### Summary

Lead is a highly toxic metal and a very strong poison. Lead poisoning usually occurs over a period of months or years. The poisoning can cause severe mental and physical impairment. Young children are most vulnerable to lead poisoning [1-2]. Lead poisoning is accompanied by an acquired deficiency of erythrocyte pyrimidine 5'-nucleotidase (P5'N). Genetically determined deficiency of P5'N enzyme was associated with chronic hemolysis, marked basophilic stippling of erythrocytes on peripheral blood smear and accumulations of intra-erythrocyte pyrimidine-containing nucleotides [3-4]. Pyrimidine-containing nucleotides are almost absent in the normal erythrocytes but it was reported that in lead poisoning, 12% of erythrocyte showed accumulation of pyrimidine nucleotides in the blood of a patient and P5'N activity was suppressed to 50% that in normal erythrocytes in lead poisoning [2]. In most of  $\beta$ -thalassemia carriers and other hemoglobin variant (Hb-E) showed marginally reduced Purine/Pyrimidine nucleotide ratios but normal P5'N-1 activity [5]. This report describes the clinical severity of lead-induced hemolytic anemia in two Indian patients with basophilic stippling associated with intra-erythrocyte accumulations of pyrimidine-containing nucleotides which mimicking hereditary P5'N deficiency.

**Keywords:** Lead poisoning; Hemolytic anemia; Basophilic stippling; Pyrimidine metabolism

### Case-1

A 30-year-old with a history of hypertension was admitted to the emergency department of King Edward Memorial Hospital in Mumbai, complaining of epigastric pain with nausea and vomiting over the previous 5 days. A patient was a born of a non consanguinity marriage belonging to the Maratha community, originating from Maharashtra, India. He had severe anemia, hyperbilirubinemia (total bilirubin 22.5 mg/dl, indirect bilirubin 11.0 mg/dl), reticulocytosis and history of one time blood transfusion. There was no family history of chronic anemia or jaundice. His USG report showed multiple small calculi in Gall bladder with hepatosplenomegaly. He referred to us with this background to investigate the cause of chronic hemolytic anemia. The peripheral blood smears showed the presence of basophilic stippling, tear drop cells, target cells, hypochromasia and aniso-poikilocytosis. Malarial parasites were not seen on peripheral blood smear as well as a malarial antigen test was negative. The measured Hb level was 4.3 g/dl, MCV 72.1 fL, MCH 18.8 pg, RDW 31.8% and a reticulocyte count of 6.0% was observed in the absence of inclusion bodies or Heinz

bodies. Heat instability test for unstable hemoglobin was negative. HPLC Analysis of hemoglobin's on the Variant Hb testing system suggests of beta thalassemia trait (Hb A2 – 4.6 and Hb F- 1.1). Molecular characterization of the beta globin gene showed the presence of heterozygous IVS 1-5 G→C mutation. Red cell enzymes such as G6PD, Pyruvate kinase and Glucose phosphate Isomerase were normal. The ratio of purine/pyrimidine nucleotides was calculated as described by Beutler and Miwa et al. [6-7]. There was a slight reduction in the Purine/Pyrimidine nucleotides ratio (1.80 and Normal range: 2.5 -3.0) but the activity of P5'N-1 was normal. Lead content in blood of patients was 53.3  $\mu$ g/dl (10-25  $\mu$ g/dl). So we emphasize that high content of lead in blood could be a possible cause of severe hemolytic anemia in thalassemia. The patient was administered an oral chelation therapy with DMSA (dimercaptosuccinic acid) 10 mg/kg 3 times daily for 5 days, and then 10 mg/kg 2 times daily for 14 days. The symptoms rapidly improved and the patient was discharged 5 days later from hospital with outpatient control by his general physician.

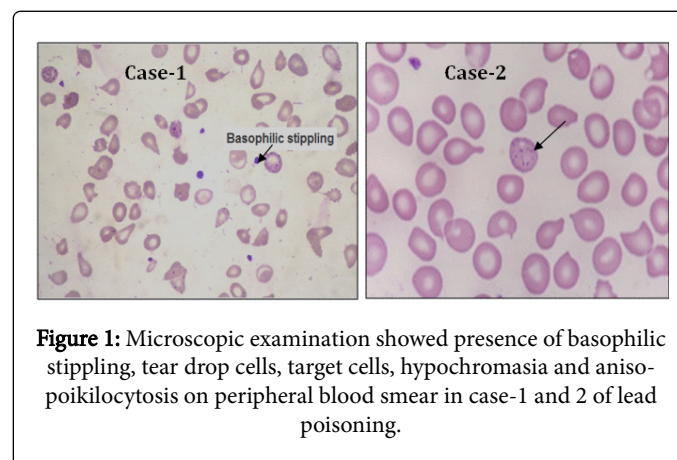
### Case-2

A 53 year old man originating from Uttar Pradesh in north India presented with a history of haemolytic anemia for one week and general physical discomfort. Initial hematological investigations were as follow; WBC  $9.1 \times 10^3$ /ml, Hb 11.2 g/dl, RBC count  $3.12 \times 10^6$ /ml. HCT 37.7%, M.C.V. 69.8 fL, M.C.H.C. 29.7%, RDW 20.1%. Microscopic examination of a stained peripheral blood smear showed severe anisocytosis, microcytosis, and basophilic stippling. This case also had mild anemia (Hb 11.3g/dl) and had reduced MCV levels. Red cell enzymes such as G6PD, Pyruvate kinase and Glucose phosphate Isomerase were normal. Heat instability test for unstable hemoglobin was negative. Biochemical analysis of hemoglobin using high-performance liquid chromatography (HPLC) showed increased levels of hemoglobin A2 (HbA2: 4.9%) and normal hemoglobin F (HbF: 0.0%). Molecular characterization of the beta globin gene showed the presence of heterozygous IVS 1-5 G → C mutation. He also had jaundice with hepatosplenomegaly. The patient did not receive blood transfusion during his lifespan. There was a slight reduction in the Purine/Pyrimidine nucleotides ratio i.e., 2.2 but P5'N-1 activity was slightly decreased. Lead content in blood of patients was 33.0  $\mu$ g/dl. Treatment with the oral heavy metal chelator, dimercaptosuccinic acid (DMSA), was started at a dose of 30 mg/kg body weight per day for 5 days followed by 20 mg/kg for 14 days. After 3 weeks, the blood lead concentration had decreased to 17  $\mu$ g/dl, anaemia and red blood cell morphology had normalized and the patient had become asymptomatic.

The clinical, hematological and biochemical data of the two cases of lead induced P5'N-1 deficiency at the time of the study are summarized in Table 1. First case had severe anemia (Hb 4.3 g/dl) and both cases had reduced MCV levels and raised Hb A2. The clinical and hematological features of both the cases of lead poisoning not distinctive, although the blood film gives a strong clue as to the diagnosis when marked red cell basophilic stippling is seen. Basophilic stippling is a constant but not specific finding in this disease, occasionally occurring in other congenital or acquired conditions, such as  $\beta$ -thalassemia trait, some hemoglobin variants, sideroblastic anemia or pyrimidine 5'nucleotidase deficiency [8-10]. The lead poisoning diagnosis ultimately depends upon the high level of lead content in blood and high concentrations of pyrimidine nucleotides and a reduced P5'N-1 activity in red blood cells [11]. The nucleotides of normal red cells consist largely of purine derivatives (which have an absorption maximum at about 260 nm), with very low levels of pyrimidine nucleotides (absorption at 280 nm). In P5'N-1 deficiency, high levels of pyrimidine nucleotides accumulate in erythrocytes, resulting in a decrease in the OD 260/280 absorbance ratio [12]. Both the cases had a similar syndrome as seen with hereditary P5'N-1 deficiency. There was a slight reduction in the Purine/Pyrimidine nucleotides ratio, but the activity of P5'N-1 in one case was normal. This is due to the high reticulocyte count (6.5%) or because P5'-1 activity is progressively inhibited until the erythrocyte lead concentration reaches 200  $\mu$ g/dl RBC at which point the activity was maximally depressed [11]. Both the patients had no history of taking any ayurvedic or traditional medicine for their high level of lead content. There was no industrial area such silversmiths, print shops, brass works, Pb battery assembly workshops, radiator repair workshops and other such workshops which exposed lead around their dwelling places. The main source of Lead exposed can suspect to be water supplies or water contamination from lead pipe and tinned eating utensils. The common practice of using a Pb-Sn (lead-tin) alloy coating the inside of copper eating utensils is considered to have potentially widespread impacts [13]. Vessels and pipe works for drinking water are also of concern but there is limited information. The majority of cases of adult lead poisoning originate from workplace exposures. Inorganic lead is absorbed from the lungs, especially in adults, or from the gastrointestinal tract, which represents the predominant exposure route in children. Lead from herbal medicines, especially from Asian countries, is an emerging source of heavy metal poisoning. The blood lead concentration correlated with the symptoms at presentation. Lead concentrations below 50  $\mu$ g/dl may cause symptoms such as asthenia, arthralgia, hypertension, headache and even infertility [14-15]. Above these concentrations abdominal colics, kidney dysfunction, haemolytic anaemia and encephalopathy may occur. Much of the toxicity of lead can be attributed to interference with calcium-mediated signalling or the distortion of enzymes and structural proteins, in this case leading to symptoms of acute porphyria by impairing enzymes of porphyrinbiosynthesis [16]. Measurement of the blood lead concentration is the mainstay of diagnosis, but careful history taking remains of paramount importance. Basophilic stippling in the blood smear suggests lead intoxication, but is non-specific. The key first step in management is to stop exposure. Medical treatment consists of chelation by substances such as DMSA or calcium EDTA [17-18]. Recently, in 2016, Aziza et al. reported a 46-year-old man of Iranian origin presented with chronic lead poisoning due to smoked 10 g of opium per week for a year and a half. He had the abdominal pain, nausea, vomiting and extravascular haemolytic anaemia with punctate

basophilic stippling on blood film. The patient underwent chelation therapy and has recovered clinically and biochemically [19]. In 2013, Muller et al. reported Bhutanese traditional medicines was the rare cause of lead poisoning in Western countries [20].

In conclusion, lead poisoning shares symptoms with other conditions and may be easily missed. The importance of a detailed drug history, including alternative medicines should be emphasized. The findings show that the hemolytic anemia and increased basophilic stippling characteristic of certain cases of lead intoxication may share a common etiology with essentially same features of the genetically determined disorder. Accumulation of lead content with heterozygous  $\beta$ -thalassemia is very uncommon and significant case of hemolytic anemia (Figure 1).



Patient ID	Case 1	Case 2
Age/Sex	30 yrs/M	53 yrs/M
WBC (X 10 <sup>3</sup> / $\mu$ l)	12.1	10.1
RBC (X 10 <sup>6</sup> / $\mu$ l)	2.29	5.4
Hb (g/dl)	4.3	11.2
HCT (%)	16.5	37.7
MCV (fl)	72.1	69.8
MCHC (g/dl)	26.1	29.7
RDW (%)	31.8	20.1
PLT (X 10 <sup>3</sup> / $\mu$ l)	178	282
Retic count (%)	6	6.5
T.Bili/ Indir. Bili (mg/dl)	22.5/11.0	7.80/7.30
Blood Transfusion	1	NO
Hb analysis by HPLC	A2=4.6	A2=4.9
	F=1.1	F=0.0
b	IVS 1-5 (G $\rightarrow$ C)	IVS 1-5 (G $\rightarrow$ C)
Pb content in blood ( $\mu$ g/dl)	53.3	33
P5'N Ratio	1.9	2.2

Clinical presentation	Multiple small calculi in gall bladder with hepatosplenomegaly	Indirect hyperbilirubinemia with hepatosplenomegaly
Normal Range: Lead (Pb) content in blood=10 to 25 µg/dl. P5'N Ratio (OD 260:OD 280)=3.0+0.52		

**Table 1:** Clinical, hematological and biochemical data of the two cases of Lead poisoning induced severe hemolytic anemia in beta thalassemia minor

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### References

- American Academy of Pediatrics Committee on Environmental Health (2005) Lead exposure in children: prevention, detection, and management. *Pediatrics* 116: 1036-1046.
- Buc H, Kaplan JC (1978) Red-cell pyrimidine 5'-nucleotidase and lead poisoning. *Clinica Chimica Acta* 85: 193-196.
- Chiarelli LR, Bianchi P, Fermo E, Galizzi A, Iadarola P, et al. (2005) Functional analysis of pyrimidine 5'-nucleotidase mutants causing nonspherocytic hemolytic anemia. *Blood* 105: 3340-3345.
- Rees DC, Duley JA, Marinaki AM (2003) Pyrimidine 5'-nucleotidase deficiency. *Br J Haematol* 120: 375-383.
- Nadkarni AH, Ghosh K, Mohanty D, Colah R (1997) Hemoglobin E and Pyrimidine 5' Nucleotidase Deficiency. *Blood* 90: 1716a-1716.
- Beutler E (1984) *Red Cell Metabolism: A Manual of Biochemical Methods* 3rd edn. Grune & Stratton, Inc, New York, NY.
- Miwa S, Luzzafato L, Rosa R, Paglia DE, Schroter W, et al. (1989) Recommended screening test for pyrimidine 5'-nucleotidase deficiency. *Clin Lab Haematol* 11: 55-56.
- Joishy SK, Shafer JA, Rowley PT (1986) The contribution of red cell morphology to the diagnosis of beta-thalassemia trait. *Blood Cells* 11: 367-374.
- Sozen M, Karaaslan C, Oner R, Gumruk F, Ozdemir MA, et al. (2004) Severe hemolytic anemia associated with Hb Volga [G27(B9)Ala? Asp]: GCC →GAC at codon 27 in a Turkish family. *Am J Hematol* 76: 378-382.
- Zanella A, Bianchi P, Fermo E, Valentini G (2006) Hereditary pyrimidine 5'-nucleotidase deficiency: from genetics to clinical manifestations. *Br J Haematol* 133: 113-123.
- Vives Corrons JL (2000) Chronic non-spherocytic haemolytic anaemia due to congenital pyrimidine 5' nucleotidase deficiency: 25 years later. *Bailliere's Best Pract Res Clin Haematol* 13: 103-118.
- Sakai T, Ushio K (1986) A simplified method for determination erythrocyte pyrimidine 5' Nucleotidase (P5'N) activity by HPLC and its value in monitoring lead exposure. *Br J Ind Med* 43: 839-844
- Needleman H (2004) Lead poisoning. *Annu Rev Med* 55: 209-222.
- Mike van Alphen (1999) Lead Poisoning in India. *LEAD Action News* 7: 1324-6011.
- Hiroyoshi F (2002) Lead, chemical porphyria and heme as biological mediator. *J Exp Med* 196: 53-64.
- Doumouchtsis KK, Doumouchtsis SK, Doumouchtsis EK (2009) The effect of lead intoxication on endocrine functions. *J Endocrinol Invest* 32: 175-83.
- Kosnett MJ, Wedeen RP, Rothenberg SJ (2007) Recommendations for medical management of adult lead exposure. *Environ Health Perspect* 115: 463-71.
- Bradberry S, Vale AA (2009) Comparison of sodium calcium edetate (edetate calcium disodium) and succimer (DMSA) in the treatment of inorganic lead poisoning. *Clin Toxicol (Phila)* 47: 841-858.
- Azizi A, Ferguson K, Dluzewski S, Hussain T, Klein M (2016) Chronic lead poisoning in an Iranian opium smoker resident in London. *BMJ Case Rep*.
- Toniolo M, Ceschi A, Meli M, Lohri A, Favre G (2011) Haemolytic anaemia and abdominal pain- a cause not to be missed. *Br J Clin Pharmacol* 72: 168-169.