A CASE REPORT : Novel homozygous missense variant in CLCN7 gene causing autosomal recessive osteopetrosis type 4 in 11 days old Saudi girl presented with neonatal hypocalcaemia.

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ABSTRACT: Osteopetrosis is a rare genetic disease that is relatively common in Saudi Arabia because of the high rate of consanguineous marriages. Hypocalcemia is a rare presentation of malignant infantile osteopetrosis (MIOP). Here we are reporting a case of autosomal recessive malignant Osteopetrosis type 4 with a novel missense variant mutation on CLCN7 gene not reported before. This patient presented with symptomatic hypocalcaemia in the age of eleven days , which is a very rare and very early presentation for this disease.

KEYWORDS : osteopetrosis , CLCN7 gene, novel missense variant , hypocalcemia

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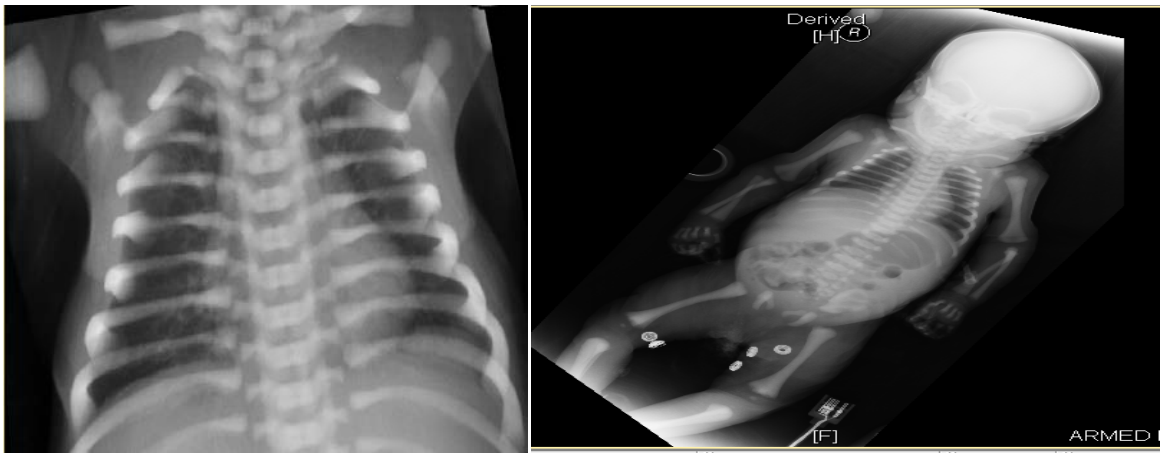
INTRODUCTION: Osteopetrosis ("marble bone disease") is a descriptive term that refers to a group of rare, heritable disorders of the skeleton characterized by increased bone density on radiographs. The overall incidence of these conditions is difficult to estimate but autosomal recessive osteopetrosis (ARO) has an incidence of 1 in 250,000 births, and autosomal dominant osteopetrosis (ADO) has an incidence of 1 in 20,000 births. Osteopetrotic conditions vary greatly in their presentation and severity, ranging from neonatal onset with life-threatening complications such as bone marrow failure (e.g. classic or "malignant" ARO), to the incidental finding of osteopetrosis on radiographs (e.g. osteopoikilosis). Classic ARO is characterised by fractures, short stature, compressive neuropathies, hypocalcaemia with attendant tetanic seizures, and life-threatening pancytopaenia. Osteopetrosis is caused by failure of osteoclast development or function and mutations in at least 10 genes have been identified as causative in humans, accounting for 70% of all cases. Diagnosis is largely based on clinical and radiographic evaluation, confirmed by gene testing where applicable.Treatment of osteopetrotic conditions is largely symptomatic, although haematopoietic stem cell transplantation is employed for the most severe forms associated with bone marrow failure and currently offers the best chance of longer term survival in this group. [(1)]

CASE REPORT: Our patient was a female newborn presented to our hospital at age of 11 days old with the concern of decreased activity and poor suckling for two days before admission. No history of fever. Parents are consanguineous. This is the first sibling to this young couple . No family history of concern. Sepsis was suspected so full septic screen was done and patient was started on broad spectrum antibiotics awaiting the result of the septic screen. CXR was done as part of full septic screen (Figure 1) . It showed increased bone density suggestive of osteopetrosis. Her laboratory

investigations showed sever hypocalcemia and thrombocytopenia. Ultrasound abdomen showed mild splenomegally. CBC: WBC 21.85 x 10^9/L, RBC 4.33 x 10^12/L, Hb 14.8 g/dL, HCT 44.1%, MCV 101.9 n, MCH 34.3 pg, MCHC 33.6 g/dL, RDW 16.4%, PLT 39 x 10^9/L , U&Es: Na 140 mmol/L, Cl 107 mmol/L, Urea 3.5 mmol/L, creatinine 45 µmol/L, Bone profile: Ca 1.06 mmol/L (4.2 mg/dl), albumin 30 g/L, Mg 0.54 mmol/L, phosphorus 2.89 mmol/L, ALP 200 U/L, corrected Ca 1.26 mmol/L(5.04 mg/dl) . The result of vit D level and parathromone level were normal. After 48 hours all cultures came negative and antibiotics were stopped.



Because of her symptomatic hypocalcaemia(lethargy, poor oral intake) , patient received one alpha drops along with high dose oral calcium supplementations. There were good response. The serum calcium gradually normalize and the symptoms of decreased activity and poor suckling improved and the patient was discharged in stable condition. Because the concern of osteopetrosis , the WES study (whole exome sequencing) was done and confirmed the diagnosis of osteopetrosis. WES identified pathogenic variants in the CLCN7 that have been linked to autosomal recessive Osteopetrosis 4 (OMIM #611490). WES identified the homozygous missense variant (c.890 T>A) p.(Val297Glu)(chr16:1506140;hg19) in exon 10 of the CLCN7. To the best of our knowledge this variant has not been described in the literature so far. Nine out of ten bioinformatic analysis tools used here predict this variant to be pathogenic.

DISCUSSION: Osteopetrosis (OPT) is a life-threatening disease caused by subnormal osteoclast function, with an incidence of 1 in 250,000 births. The disease usually manifests in the first few months of life with macrocephaly and frontal bossing, resulting in a characteristic facial appearance. The expanding bone encroaches on neural foramina, leading to blindness, deafness, and facial palsy. Complete visual loss invariably occurs in all untreated patients, and hearing loss is estimated to affect 78% of patients with OPT. Tooth eruption defects and severe dental caries are common. Calcium feedback hemostasis is impaired, and children with OPT are at risk of developing hypocalcemia with attendant tetanic seizures and secondary hyperparathyroidism. The most severe complication of OPT, limiting survival, is bone marrow insufficiency. The abnormal expansion of cortical and trabecular bone physically limits the availability of medullary space for hematopoietic activity, leading to life-threatening cytopenia and secondary expansion of extramedullary hematopoiesis at sites such as the liver and spleen[(2)].Disturbances of calcium homoeostasis have been well described in malignant infantile osteopetrosis (MIOP). The first description may have been provided by Avery et al., who described a baby born with osteopetrosis in 1956 that was a “bit twitchy” at 6 days of age[(3)]. Srinivasan et al. reported symptomatic hypocalcemia that developed in the first month of life in 8 infants from 6 different families [(4)]. Our patient represented very early at age of eleven days old with lethargy , decreased activity , poor oral intake and poor suckling. There was no fever but because of being eleven days old with the above mentioned symptoms, sepsis was considered and full septic investigations was done including chest X ray. Parenteral antibiotics were started awaiting the result of septis screen. All the results of septic screen were negative and the antibiotics were stopped soon.

Surprisingly the chest X ray showed very significant increased in the bone density highly suggestive of osteopetrosis. The rest of investigations increased the possibility of osteopetrosis. Complete blood cound showed significant thrombocytopenia with platelet count of 39 x 10^9/L. The bone profile confirmed the significant hypocalcemia . Abdominal ultrasound confirmed spleenomegally . All these finding was pointing to osteopetrosis as the causative disease for this symptomatic hypocalcemia. Other causes of neonatal hypocalcemia were excluded. Osteprtrosis is a very rare cause of neonatal hypocalcemia but still to be considered as cause of neonatal hypocalcemia especially in our community where there is significantly high percentage of consanguinity. According to our knowledge our case is the first case in our area presented with symptomatic neonatal hypocalcemia as the initial presentation of osteopetrosis.There is a lot of reported cases of osteopetrosis from the Arab Gulf area. Abdel-Al 1994 reported nineteen Arab children including six boys and 13 girls in ten sibships as having osteopetrosis over a 5-year period in various hospitals in Kuwait[5]. While Mahdi 1988 reported ten cases from Saudi Arabia [6] . It seems also that the prevalence is higher than previously reported. Consanguinity, which is high in this group and in our community, explains the high prevalence of autosomal recessive osteopetrosis. Autosomal recessive osteopetrosis-4 (OPTB4) is caused by homozygous or compound heterozygous mutation in the CLCN7 gene on chromosome 16p13. An autosomal dominant form of osteopetrosis (OPTA2) is also caused by mutation in CLCN7. CLCN7 encodes the Cl−/ H+ exchanger CLC-7 chloride channel of the osteoclast membrane, which is required for acidification of the resorption lacunae[7]. Mutations in the CLCN7 affect the function of osteoclast-mediated extracellular acidification, resulting in the disturbed dissolution of the bone inorganic matrix and a series of clinical features [8]. Mutations in the CLCN7 are responsible for about 75% of cases of autosomal dominant osteopetrosis, 13% of cases of autosomal recessive osteopetrosis, and all known cases of intermediate autosomal osteopetrosis[9]. Our case report identified novel homozygous missense variant of CLCN7 gene not identified before. WES identified pathogenic variants in the CLCN7 that have been linked to autosomal recessive Osteopetrosis 4 (OMIM #611490). This variant has not been described in the literature so far. Allele frequencies in the general population have not been documented . Nine out of ten bioinformatic analysis tools used here predict this variant to be pathogenic. However , another nucleotide change (c.889 G>A) in the same codon that causes another aminoacid change, namely (Val297Met) reported in Indian patients with autosomal recessive infantile malignant osteopetrosis by Phadke et al 2010.[10] Our area in the southern region of Saudi Arabia has very high prevalence of consanguinity and we think the prevalence of osteopetrosis is underestimated and we need to increase the awareness of the physicians to report these cases. Doing genetic testing to this group of patients may reveal novel mutations not reported before. Also we recommend that osteopetrosis should be included in the differential diagnosis of neonatal hypocalcemia .

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