CASE REPORT

'TEST-NEGATIVE ANGELMAN SYNDROME' WITH THYROID DYSFUNCTION: A RARITY BUT A REALITY!

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Angelman Syndrome (AS) is believed to be a complex neuro-developmental genetic disorder that is often described clinically by the presence of behavioural uniqueness and movement disorders; in addition to having developmental delay and speech impairment. Genetic factors have been linked to the syndrome's aetiology in 90% of cases, although in 10% cases, an unidentified genetic mechanism accounts for the classic phenotypic features of AS. Angelman Syndrome in general or with associated thyroid dysfunction, have never been reported from Pakistan. This is the first ever case report from Pakistan reporting a rare case of clinically diagnosed AS with associated thyroid dysfunction in the presence of normal molecular genetic testing (DNA methylation test and UBE3A gene sequencing). In future, clinicians should make efforts in documenting similar cases with associated clinical profiles from our part of the world, thereby contributing to the local and regional epidemiology of these syndromes.

Keywords: Angelman syndrome, Angelman-Like syndrome, 'Test-negative Angelman Syndrome', DNA methylation test, UBE3A gene analysis, thyroid dysfunction

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INTRODUCTION

Angelman Syndrome (AS) is believed to be a complex neuro-developmental genetic disorder that is often described clinically by the presence of some form of behavioural uniqueness and movement disorders; in addition to having functionally severe developmental delay & speech impairment.[1–4] The syndrome has been mainly linked to the presence of chromosomal mutations involving; deletions in maternal chromosome 15q11–q13, which accounts for 70–80% cases of AS, abnormalities in UBE3A gene sequencing and disruptions in genomic imprinting patterns.[5–7]

The true incidence of AS still remains unknown and varies in range from 1 in 10,000 to 1 in 40,000 in different populations.[1,5] Even though genetic factors have been linked to the syndrome's aetiology in 90% of cases, a presently unidentified genetic mechanism still accounts for the remaining 10% of cases of AS[7], making them resistant to diagnostic testing[7,8]. Diagnosis of AS is mainly clinical, based on the updated clinical diagnostic criteria of AS[6], and is often augmented with various diagnostic modalities including DNA methylation tests and fluorescent in situ hybridization (FISH) techniques[3,9].

Treatment of AS is often symptomatic with efforts being made to improve the quality of life through physical therapy and rehabilitation.[9,10] AS or Angelman-like syndromes, in general or with associated thyroid dysfunction, have never been reported from Pakistan. Present global literature in this regard, is limited to only three reports retrieved via PubMed, describing thyroid dysfunction and its association in confirmed cases of AS.[11,12] Recently published review on Angelman-like syndromes[13], highlights the possibility of findings these syndromes in some of the clinically diagnosed 'test-negative AS'. However associated thyroid dysfunction in these syndromes has not been delineated as yet. This is the first ever case report from Pakistan reporting a rare case of clinically diagnosed AS with associated thyroid dysfunction in the presence of normal DNA methylation test and UBE3A gene sequencing.

CASE REPORT

An 18 years old female patient, presented to our department of Medicine at Fauji Foundation Hospital (FFH) Peshawar for evaluation of her thyroid status. She had been a known case of clinical hypothyroidism that was being treated with *Thyroxine Sodium* (50 mcg) at the dose of 5 mcg/kg/day since the age of 6 years. Her TFTs were serially monitored every 3–4 months; however TFTs from April 2013 showed evidence of subclinical hyperthyroidism and the patient was admitted to medical ward for further management (Table-1).

On initial assessment, her chief complaints included constipation, generalized body aches, fatigue and non-specific allergies that had become more prominent since past 3–4 months. She had been taking iron and folic acid supplements for past 7 years for her chronic non-settling anaemia and had history of multiple admissions to this hospital since the age of 6 years. In December 2006 she presented to the dental clinic with complaints of tooth ache. Diagnosis of broken down root (BDR) # 46 was made and the tooth was subsequently extracted. She also had history of recurrent attacks of pharyngitis since January 2009 that had been treated with appropriate antibiotics and analgesia.

According to patient's father, she was born at term as a healthy baby; however she soon developed feeding difficulties and was noted to have truncal hypotonia that became more prominent by the age of 6 months. She displayed significant delay in achieving early developmental milestones according to her age and had persistent social smiling at 1–3 months of age. She was also reported to have low Intelligent Quotient (IQ) during her preschool and early school years, though with time she displayed forward yet delayed progression in the development of normal gross-motor skills.

There was no past history of any seizure attack. Family history was insignificant for any hereditary disease; siblings included two sisters and two brothers with normal development and functioning. Menstrual history was unremarkable; menarche was achieved at the age of 13 years.

Examination revealed female patient of stated age, sitting comfortably on bed in a happy demeanor (Figure-1). Most interesting and noteworthy examination finding in the patient included behavioural uniqueness with frequent and inappropriate episodes of laughter while answering questions about her illness. This was accompanied by speech impairment including minimal use of words and use of non-verbal communication skills like head nodding during conversation. She was also noted to develop tremulous movements of hands that were more pronounced when attempting to perform a task. Mild ataxia of gait was also noted including; wide based gait and unsteadiness while walking. These findings, in view of her past medical history, strongly raised the suspicion of the rare AS in this patient. Based on Updated Consensus for Diagnostic Criteria of AS 2005[6], patient underwent an array of baseline laboratory investigations and radiological analysis including computed tomography (CT) scan of brain. Laboratory parameters showed evidence of normocytic hypochromic anaemia with decreased total red blood cell (RBC) count and RBC indices lying at lower levels of reference range. Findings were suggestive of Anaemia of Chronic disease – ACD. In addition, serum erythrocyte sedimentation rate (ESR) and alkaline phosphatase (ALP) levels were also found to be elevated. CT scan was essentially normal associated with only mild to moderate cortical and subcortical atrophy. Nonspecific EEG changes were noted, with evidence of delta (3 Hz) waveform activity, more prominently in the occipital and temporal regions, indicating the possibility of gelastic seizure activity. The results were in good agreement with the diagnostic criteria of AS, based on history and laboratory findings in AS.[6] A standard general physical examination including a complete neurological examination was performed in the patient. In addition, anthropometric measurements were also recorded indicating hypertelorism, telecanthy and growth restriction (Table-2). Dental findings were unremarkable with no carious lesions and diastemas between the teeth. Some food particles were found in the sulci showing the possibility of poor swallowing ability.

A patient with such developmental history and clinical features consistent with AS[6], warranted confirmation with initial DNA methylation test. Since the facility was not available in Pakistan, patient's blood samples were sent to Molecular Genetics Laboratory, Seattle for the initial DNA methylation test. Molecular genetic report indicated normal methylation pattern within Prader-Willi/Angelman critical region.

In light of such finding, patient's DNA samples were further analysed via UBE3A gene sequencing from, Klinikum Stuttgart Institute Germany, which further reported no mutation in UBE3A gene sequence. Therefore, AS due to UB3EA mutation could not be confirmed in this patient. The results were however interpreted with a possibility of 'test-negative AS', found in 10% of individuals with classic phenotypic features of AS, due to a presently unidentified genetic mechanism.

Table-1: Thyroid profile of our patient over period of 2 years, 2011–13

Thyroid Function Tests	Reference Range (Adult Female)	Results			Interpretation
		08-04-11	10-05-12	15-04-13	
Serum T4	4.5–10.9 µg/dL	0.66 µg/dL	1 µg/dL	9.4 µg/dL	Levels of TSH trending downwards; however, latest evidence suggestive of Subclinical Hyperthyroidism
Serum T3	80–200 ng/dL	76 ng/dL	103 ng/dL	159 ng/dL	
Serum TSH	0.4–5.0 microU/mL	50.87 microU/mL	18.1 microU/mL	0.412 microU/mL	

T4 - Thyroxine, T3 - Tri-iodothyronine, TSH - Thyroid Stimulating Hormone, µg/dL - microgram per deciliter, ng/dL - nanogram per deciliter, microU/mL - microunits per milliliter

Table-2: Physical Examination and Anthropometric measurements in our patient

Examination Parameters	Values	Interpretation
Weight (Percentile)	50 kg (25th percentile)	Weight at 25th percentile and combined with height, at 18 years indicates growth restriction
Height (Percentile)	152.4 cm (>3rd percentile)	Height between 3- 10 percentile and combined with weight, at 18 years indicates growth restriction
Body Mass Index (BMI) – Percentile	21.52 kg/m^2 (50th percentile)	Normal range between 18.5 - 24.9 kg/m^2
Inter-pupillary distance	70 mm	Hypertelorism; Normal Inter-pupillary distance range, 60-62 mm
Inter-canthal distance	40 mm	Telecanthy; Normal Inter-canthal distance range, 30-31 mm
Head Circumference (Percentile)	54 cm (< 50th percentile)	Between 2 - 50 percentile at 18 years, no evidence of microcephaly
Breast development	Tanner Stage 5	Areolar recesses to the general contour of breast, pubic hair were adult in quantity along with horizontal (feminine) pattern of distribution
Pubic hair development		

A – Telecanthy & Hypertelorism, B – Inappropriate bursts of laughter while answering questions, C – Speech impairment with minimal use of words, D – Non-verbal communication skills like head nodding, E & F – Overall happy demeanor with frequent smiling and easily excitable personality (Photo-credits: Courtesy of Dr Mahrukh Ayesha Ali, West Cumberland Infirmary Whitehaven, UK)

DISCUSSION

AS was first delineated as a separate clinical entity by Dr. Harry Angelman in 1965.[7,14] He recognized similar consistent features of AS in three unrelated children, thus labelling them as 'the puppet children'.[14,15] Later on, additional cases of 'Happy Puppet Syndrome' were reported by Bower and Jeavons in 1967[16], and detailed historic course of AS was subsequently described by Williams and Frias in 1982.[7]

In addition to the consistent findings that have been reported in 100% cases of AS, seizure activity and EEG abnormalities have been reported as frequent features in >80% cases of AS.[6] Recently a case report from Sri Lanka[7] reports the presence of extensor and flexor spasms, in a patient with AS, with EEG findings not corresponding to any previously reported characteristic EEG pattern[17]. In our case, non-specific EEG changes were noted that were in good agreement with the reported finding of having more prominent EEG abnormalities in patients with chromosome 15 mutations, which came out to be negative in our case.[17]

Presence of structurally normal brain finding on Magnetic Resonance Imaging (MRI) or CT scan, may also be a useful criteria in diagnosis of AS. However, some reports have documented the presence of abnormal myelination in patients with AS.[18] In our case mild to moderate cortical and subcortical atrophy was appreciated, which was in good agreement with the similar findings documented by other case reports.[7,11]

Studies from the past[11] have reported the presence of thyroid dysfunction in patients with AS, linking them to the possibility of abnormal body metabolism and use of anticonvulsants in such patients.[11] In this regard, some studies have related the presence of disrupted hypothalamic-pituitary-thyroid axis in patients with AS to chromosome 15 mutations[11], while others have proposed thyroid dysfunction as a result of imprinting mutations in genes involved in thyroid metabolism[19].

Interestingly in our case, no DNA methylation abnormalities and UBE3A gene mutation was found. Likewise serum ALP levels and ESR were also found to be elevated on initial laboratory analysis. Such findings, in presence of thyroid dysfunction and associated clinical features of AS, were likely to be attributed to an associated immune mediated process, as described by few other reports as well.[11,12] Likewise, presence of ACD along with history of non-specific allergies and recurrent pharyngitis, in our patient, further supports this proposition! In this regard, a report from India

documents the presence of microcytic hypochromic anaemia and elevated serum ESR levels, in a patient with AS.[20]

The updated consensus for diagnostic criteria in AS has documented a variety of associated symptoms, accounting for 20–80% cases of AS.[6] In our case, these associated symptoms included; constipation, sleep disturbances, poor swallowing ability, truncal hypotonia during infancy, wide-based gait and abnormal fascination to crinkly items like paper. Although some of these overlap with features of hypothyroidism, thyroid dysfunction in particular has not been described as part of AS. However, as in our case, presence of similar symptoms should necessitate the need of prompt analysis for thyroid dysfunction, in suspected cases of AS.

Recently a detailed review on Angelman-like syndromes[13], highlights some of the key features unique to these syndromes. An insight to these syndromes is of vital importance, when suspecting any patient with AS, since many features of AS are also shared by these syndromes. However in our case, features unique to Angelman-like syndromes were not found thereby, significantly increasing the probability of having 'test-negative AS', as the likely diagnosis in our case.

CONCLUSIONS

Confirmatory diagnosis of AS or other similar syndromes cannot be made without genetic testing; however, a variety of unique clinical features distinguishes these syndromes from other clinical disorders. It is therefore imperative, that clinicians from our part of the world should increase awareness about Angelman and other related syndromes, and efforts should be made in documenting similar cases with associated clinical profiles; thereby contributing to the local and regional epidemiology of these syndromes.

AUTHOR'S CONTRIBUTION

MUA was involved in; management of patient along with conception, drafting and design of the work. AM and MAA; were involved in management of patient in addition to revising the manuscript critically for important intellectual content. All the authors read the manuscript and gave final approval of the version to be published, and will be accountable for all aspects of the work in ensuring the accuracy or integrity of any part of the work published.

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