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Research paper

Exome Sequencing of Suspected Tuberculoma Patient

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ABSTRACT

We have carried out a case study of a suspected tuberculoma patient. Besides clinical data analysis, we carried out exome sequencing of this patient to have a better insight into diagnosis. For exome sequencing, the genomic DNA was extracted and purified from the blood of the patient followed by whole exome sequencing using DNBseq's next-generation sequencing technology. The exome sequence data was analyzed to find out the genes containing rare and pathogenic variants. We observed the "KCNG4", "PABPC3", and "TAT "genes harbor pathogenic variants. Bioinformatic analysis of ENSEMBL, GNOME, and COSMIC databases revealed the involvement of these SNVs in small ring-enhancing lesions in the middle cerebellar peduncle region of the central nervous system. These findings provided new insight related to the clinical condition of this patient.

KEYWORDS: KCNG4; PABPC3; TAT; Cerebellar peduncle

INTRODUCTION

Tuberculosis (TB) is a contagious bacterial infection caused by Mycobacterium tuberculosis. It mainly affects the lungs but can also affect other body parts, such as the brain, spine, and kidneys. TB spreads through the air when an infected person coughs, sneezes, or talks, and another person inhales the bacteria (https://www.cdc.gov/tb/index.html).

Symptoms of tuberculosis can include persistent cough, chest pain, fever, night sweats, weight loss, and fatigue. TB can be diagnosed through various tests, including skin tests, blood tests, sputum tests, and chest X-rays. Treatment for tuberculosis typically involves a combination of medications taken over six to nine months. TB is a serious and potentially life-threatening disease, especially for people with weakened immune systems. However, with early diagnosis and appropriate treatment, most people with TB can be cured.

On the other hand, tuberculomas or tuberculous granulomas are well-defined focal masses that result from *Mycobacterium tuberculosis* infection and are one of the more severe morphological forms of tuberculosis. Tuberculomas most commonly occur in the brain (CNS tuberculosis) and in the lung. Tuberculoma is a type of tuberculosis infection that occurs in the brain [1-3]. It is a mass or a lesion that develops in the brain due to infection caused by the causative agent of tuberculosis i.e. *Mycobacterium tuberculosis*. It is a rare complication of tuberculosis and typically occurs in people who already have a weakened immune system. Symptoms of tuberculoma can include headaches, seizures, confusion, and difficulty with movement. Treatment for tuberculoma typically involves a combination of medications to target the tuberculosis bacteria, and in some cases, surgery may be necessary to remove the mass or lesion [4,5].

Here, we present a case study of a male patient with suspected tuberculoma. In

January 2011, the patient experienced a loss of balance and sought advice from a diabetologist who suspected a stroke due to diabetes and recommended consulting a neurologist. After undergoing several MRIs and other clinical tests, the patient was diagnosed with suspected brain tuberculosis (Tuberculoma). Clinical tests such as CBC, biochemistry, electrolytes, C-reactive protein, gram staining, LFT, CSF, ESR were normal. The MRI revealed edema extending into the medulla and small ring-enhancing lesions in the middle cerebellar peduncle, likely representing tuberculomas. Based on the presumptive diagnosis of tuberculoma in this patient, the clinician prescribed a treatment plan consisting of the following medications: Aspirin, Ethambutol, Humulin-N Insulin, Humulin-R Insulin, Isoniazid, Lipiget, Pyrazinamide, Pyridoxine, and Rifampicin.

After initiating the TB treatment, the patient's condition worsened, and in February 2011, the patient was hospitalized. Despite this, the clinician continued with the TB treatment for almost a year. By the end of 2011, the patient had become disabled. Eventually, the clinician discontinued the TB treatment as it was not proving to be effective. The patient was given steroids a few times, with the last administration occurring in December 2019. The patient gradually lost his ability to speak and is now completely bedridden.

Hence presumptive diagnosis was not effective in this case. The presumptive diagnosis is a diagnosis that is made based on the patient's medical history, symptoms, and initial examination, without any confirmatory diagnostic test. It is a preliminary diagnosis that is made to guide treatment and further investigation. The term "presumptive" is used because it is based on the clinician's assumption that the diagnosis is most likely correct based on the available information, but it has not been confirmed by specific diagnostic tests [6-8]. Presumptive diagnosis is often used in situations where waiting for the results of confirmatory tests could delay necessary treatment and potentially harm the patient. Once the confirmatory diagnostic tests are performed, the diagnosis can be confirmed, refined, or ruled out, and the treatment plan can be adjusted accordingly [9,10].

A definitive diagnosis is made after confirmatory diagnostic tests have been performed and a specific medical condition or disease has been identified. Unlike a presumptive diagnosis, a definitive diagnosis is based on conclusive evidence obtained from diagnostic tests, such as laboratory tests, imaging studies, or biopsies. A definitive diagnosis is more accurate and reliable than a presumptive diagnosis and allows for more targeted and effective treatment.

MATERIALS AND METHODS

To better understand the genetic basis of the pathology in this patient exome sequence analysis was proposed [11]. All procedures involving human participants were according to the ethical standards of the Independent Ethics Committee, Mohammad Ali Jinnah University, Karachi, Pakistan.

For whole exome sequencing, blood samples of the patient were obtained. The genomic DNA was extracted from the blood by GJC Genomic DNA purification kit (Gene Janch Center, Karachi, Pakistan). The purified genomic DNA sample of the patient was subjected to exome sequencing. The whole exome sequencing was performed using DNBseq technology (BGI, Shenzhen, China). The resultant exome sequences were analyzed by the following programs; FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), BWA [12], SAM

Tools [13], GATK [14], Variant Effect Predictor (VEP) [15] and Annovar [16]. The exome sequence data were aligned to the human reference genome (NCB137/hg19). The SNVs and InDels obtained by HaplotypeCaller command of GATK were annotated by Annovar program. The annotated SNVs and InDels were characterized by dbSNP [17], ClinVar [18] and the 'Catalogue of Somatic Mutations In Cancer' (COSMIC) [19] databases.

RESULTS

Detailed bioinformatics analysis of exome sequence data of this patient revealed pathogenic mutations in a few genes including *KCNG4*, *PABPC3*, and *TAT* genes (Tables 1 and 2). The COSMIC database showed that these mutations are involved in small ring-enhancing lesions in the middle cerebellar peduncle region of the central nervous system (Table 3). These findings correlated with the clinical data of the patient.

Table 1: Deleterious InDels in the exonic regions detected by COSMIC database in the tuberculoma patient

Chr	Start	End	Ref	Alt	Gene	Exonic Function	MAF	COSMIC DB annotation
chr 10	73240990	73240998	CCAGTCTCT	Del	FAM149B1	Nonframeshift deletion	0.01	(liver; lung)
chr 17	47379200	47379200	-	A	EFCAB13	Frameshift insertion	0.01	(liver; lung)
chr 2	1.7E+08	1.7E+08	-	T	FASTKD1	Frameshift insertion	0.008	(liver; lung)
chr 5	65610905	65610907	CTC	-	TRIM23	Nonframeshift deletion	0.008	(liver: lung)
chr 4	1.4E+08	1.4E+08	GCTGCTGCTGC	-	MAML3	Frameshift deletion	0.007	(liver; lung)

Table 2: Deleterious nonsynonymous SNVs in the exonic regions detected by the COSMIC database in the tuberculoma patient.

Chr	Start	End	Ref	Alt	Gene	MAF	COSMIC_DB annotation
chr16	84223013	84223013	C	T	KCNG4	0.09	(central_nervous_system)
chr16	71576283	71576283	G	A	TAT	0.03	(central_nervous_system)
chr11	55827639	55827639	C	T	OR5L2	0.07	(central_nervous_system)
chr13	25096730	25096730	G	A	PABPC3	0.06	(central_nervous_system)

Table 3: Pathogenic score of deleterious missense SNVs as predicted by FATHMM.

Gene	Genomic mutation ID	AA mutation	FATHMM prediction
KCNG4	COSV57582654	p.C255Y	Pathogenic(Score 0.99)
TAT	COSV63532218	p.P45S	Pathogenic (Score 0.96)
OR5L2	COSV65723417	p.R141C	Neutral (Score 0.00)
PABPC3	COSV55796540	p.E178K	Pathogenic (Score 0.86)

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