Original Article

Evaluation of BRAF v600 Mutation by Single Strand Conformation Polymorphism-PCR (SSCP-PCR) in Patients with Interstitial Lung Disease

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Abstract

Background: Many studies demonstrating a high predominance of pulmonary disease among individuals with interstitial lung disease (ILD), genomic examinations have recognized and initiated mutations in proto-oncogene B-Raf (BRAF) among patients with lung malignant growth. To support our hypothesis of being ILD could show a more vital event for framing into bronchogenic threat BRAFV600E change was examined. **Objectives:** To inquire about the frequency of BRAF (V600E) mutation in ILD with the possible evaluation of the presence of BRAF V600 mutation and such parameters as patient's age, gender, and histopathological type. **Materials and Methods:** From January 2017 to April 2019, a review study was led in a few confidential labs and the Teeba respiratory focus in Hilla city, Babylon region. Sixty patients with interstitial lung issues' clarifying data were recuperated, formalin-fixed paraffin-embedded (FFPE) tissue portions were furthermore collected, all of the results were surveyed by three expert histopathologists, and the last attestation of the examination was done. Equivalent number (60) of healthy lung tissues were also involved and used as control tests for polymerase chain reaction review. **Results:** Only two (3.3%) of ILD tests harbored BRAF V600E change, all of them were between 41 and 50 years and were the usual interstitial pneumonitis (UIP) type. **Conclusions:** BRAF V600E mutation likely could be identified in ILD especially UIP. Prognosis of such patients with mutated BRAF gene should be observed.

Keywords: BRAF V600E, interstitial lung disease, lung carcinoma, SSCP-PCR

Introduction

Interstitial lung diseases (ILDs) were gathering of disorders (in excess of 200 sorts) of obscure or known etiologic causes that cause scarring (fibrosis) of the lungs. Because of the scarring, the lungs become stiff, making it hard to breathe, and get oxygen into the bloodstream. ILD-caused lung damage is frequently irreversible and worsens over time.[1]

These widespread infiltrating pulmonary issues are ordinarily portrayed by the nearness of aggravation and modified interstitial tissue of the lung, and explicit types of ILDs could be separated from each other when clinical information, images of radiological examination, and histopathologic examination (if lung biopsy is required) are consolidated to arrive at a sure diagnosis.[2]

ILDs are traditionally partitioned into four classifications: (i) ILDs of well-identified causes, for example, connective tissue disorders (CTDs), environmental, or drug-induced; (ii) idiopathic interstitial pneumonias; (iii) sarcoidosis; and (iv) different types of ILDs, for example, lymphangioleiomyomatosis, Langerhans' cell histiocytosis, alveolar lipoproteinosis or eosinophilic pneumonia.[3] Idiopathic pulmonary fibrosis (IPF), sarcoidosis, hypersensitivty pneumonitis (HP), ILD as an appearance of CTD, drug-induced, and pneumoconiosis are the most prevalent ILD.[4]

Regardless of being an uncommon illness, the ongoing Global Burden of Disease Study detailed that, somewhere

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in the range of 1990–2013, there was an 86% expansion in ILD-related long stretches of years of life lost (YLL); as a result, ILDs were incorporated, just because it is among the main 50 reasons for worldwide YLL.[5]

Immunosuppressive therapies are utilized to treat different types of ILD.[6]

ILDs are portrayed as ceaseless collection and quick expansion of separated fibroblasts in districts of rehashed epithelial damage alongside expanded protection from apoptosis.[7]

Inflammation can prompt numerous hereditary modifications influencing cell development, separation, and endurance, including the mutation of genes that suppress tumors (such as p53), the activation of oncogenes, and the turnover of genes that cause apoptosis.[8]

The proto-oncogene B-Raf (BRAF) gene on chromosome 7 (7q34) encodes the BRAF protein, which shares in the mitogen-actuated protein kinase (MAPK)/extracellular signal-regulated kinases signaling pathway. This pathway directs significant cell capacities including cell development, separation, multiplication, senescence, and apoptosis.[9]

More than 40 distinct changes have been recognized in the BRAF quality in human disease, 90% of BRAF transformations are represented by a single-base change from thymine to adenine at position 1799. At buildup 600, this missense transformation in exon 15 results in a change that replaces valine with glutamine (V600E).[10]

BRAF demonstrated to be transformed into many diseases (40%–70% of dangerous melanoma, a normal of 45% of papillary thyroid malignant growth, and of colorectal tumors and has likewise been recognized in ovarian, breast, and lung malignancies).[10]

Materials and Methods

From January 2017 to April 2019, a retrospective study was conducted in several private laboratories and the Teeba respiratory center in Hilla city, Babylon province. Sixty patients with interstitial lung issues' elucidating information were recovered, and FFPE tissue segments were additionally gathered, every one of the outcomes was assessed by three master histopathologists, and the last affirmation of the analysis was finished. A comparable number (60) of ordinary lung tissues were additionally involved and utilized as control tests for the polymerase chain reaction (PCR) study.

Our goal for this study was to evaluate the expression of BRAF V600 mutation in a group of patients with ILD without any evidence of associated lung carcinoma.

Molecular study

FFPE tissue DNA extraction

FFPE tissue area tests were separated utilizing NEXprep FFPE tissue kit, Genes Laboratories, Seoul, South Korea. The extraction was accomplished by the protocol of the manufacturer. Briefly, xylene was utilized to expel the paraffin waxes and cleaned out by total ethanol. At that point, genomic DNA was extricated with Proteinase K, and aliquots from the removed DNA tests were measured utilizing NanoDrop spectrophotometer (Massachusetts, United States). At long last, the examples were put in −20C° for further use in single-strand conformation polymorphism (SSCP)-PCR tests.

Single-strand conformation polymorphism

It is characterized as a conformational distinction of single-stranded nucleotide successions of the corresponding length as initiated by contrasts in the arrangements under certain test situations. This characteristic permits the arrangements to be recognized by methods for gel electrophoresis, which isolates parts as indicated by their various conformations.[11]

SSCP-PCR was carried out for recognition of V600E change in exon 15 of the BRAF gene from tissue tests of ILD and typical lung tissue tests. The technique was completed by following the SSCP-PCR by Kobayashi *et al*.[12] and Karbel *et al*.[13]

Statistical analysis

Analysis of statistical data was cultivated utilizing SPSS (Chicago, Illinois, United States) adaptation 18. Outright factors were displayed as frequencies and rates. A *P* value of equivalent or under 0.05 was assigned as noteworthy worth.

Results

The clinical viewpoints for patients with ILDs associated with this investigation are outlined in Table 1. Among 60

Table 1: Clinical and pathological features of patients with ILD (no.=60)

Clinical and pathological variables	Frequency and percentage
Gender	
Male	32 (53.3%)
Female	28 (46.7%)
Age	
30–40	7 (11.6%)
41–50	16 (26.7%)
51–60	25 (41.7%)
61–70	9 (15%)
71–80	3 (5%)
Histopathological types	
NSIP	27 (45%)
UIP	17 (28.3%)
COP	6 (10%)
Drug-induced	6 (10%)
HP	4 (6.7%)
BRAF (PCR)	58 (96.7%)
Wild mutant	2 (3.3%)

COP: cryptogenic pneumonitis, HP: hypersensitivity pneumonitis, NSIP: nonspecific interstitial pneumonitis, UIP: usual interstitial pneumonitis

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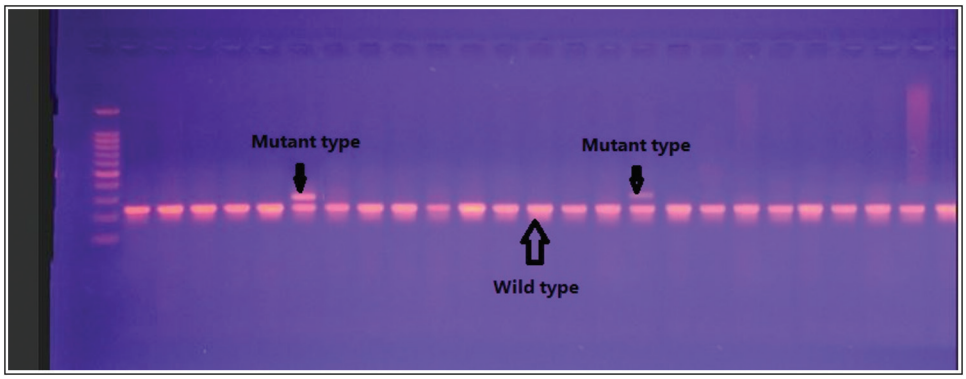

Figure 1: Agarose gel electrophoresis that demonstrates the SSCP-PCR item investigation for identification of BRAF^{V600E} mutation in exon 15 of tissue tests of ILD

Table 2: Pathological and clinical features of patients with mutant BRAF^{V600E} tumors (no. = 2)

Patient	BRAF mutation	Age	Sex	Histopathological type
1	Positive	44	M	UIP
2	Positive	50	M	UIP

M: male, UIP: usual interstitial pneumonitis

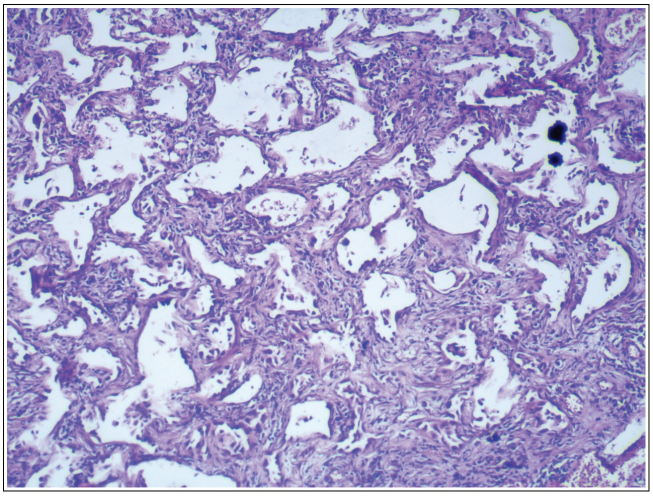

Figure 2: Usual interstitial pneumonia (UIP) shows interstitial fibrosis with honeycomb changes, and foci of fibroblastic proliferation and inflammation

instances of ILD, two (3.3%) appeared to have BRAF V600E change in exon 15 in contrast with 60 specimens of pulmonary tissues of normal histology, which uncovered just BRAF of wild sort utilizing SSCP-PCR [Figure 1].

Specific clinical highlights for mutant BRAF patients were referenced in Table 2. All ILDs with mutant BRAF quality were of usual interstitial pneumonitis (UIP) histopathological type [Figure 2] (2 of 17 instances of UIP, 11.8%).

BRAF wild-type quality was identified in 58 (96.7%) of ILD tissue samples, 30 were men and 28 were women, and their ages were between 30 and 80 years.

A nonsignificant relationship was found between wild and mutant BRAF cases with respect to the sexual orientation, age, and histopathological sorts of ILDs ($P > 0.05$).

Discussion

A prompt link between ILDs and lung cancer had been reported and published for the first time in 1939; therefore, the role of pulmonary fibrosis within the pathogenesis of tumors has been investigated since that point,[14] whereas alternative studies rumored that lung cancer incidence in patients with ILD was higher and patients with ILD might have a high risk of lung cancer development[15] that can be 3.5–7.3 times higher in ILD, with carcinoma prevalence calculable at 10%–20% in ILD and >15% of ILD patients doubtless to die from lung cancer.[16] Many common features within the pathogenesis of ILDs and carcinoma are determined throughout the last years. Highlights of pneumonic fibrosis speak to pathogenic instruments as those followed by disease cells including boundless cell increase and cell everlasting status quick migration, which describes malignant growth metastasis.[7]

In this regard, we aimed in this research to estimate the prevalence of BRAF (V600E) mutational status in ILD as an effort to link the role of such mutation in pulmonary fibrotic diseases with the event of lung cancer that could develop later on in those patients.

Toward this, we tend to study BRAFV600E mutations utilizing molecular test depending on SSPC-PCR technique, we found such mutation present in a pair of 60 cases (3.3%) of ILD, all of them were of UIP (2 of 17 cases with UIP, 11.8%). Indeed, there have been no alternative studies regarding BRAF V600E mutation in ILDs only to

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compare our results with; therefore, our study may be the first one during this issue.

The patients enclosed during this study were with a mean age of 53.4 years, SD of 9.9, and vary from 30 to 80 years, and these results were the same as stated by Turkish and Indian articles.[17,18]

The males represent 53.3%, whereas the females represent 46.7% of registered cases in our study; this was the same as the results of previous survey.[18]

Regarding the pathological forms of ILD concerned during this study, nonspecific interstitial pneumonitis was the foremost frequent (n = 27, 45%) followed by UIP (n = 17, 28.3%), cryptogenic pneumonitis (n = 6,10%), drug-induced pneumonitis (n = 6, 10%), and HP (n = 4, 6.7%); these results were totally different from Singh *et al*.'s[18] study whom incontestable that hypersensitivity pneumonitis is with the highest frequency (n = 513, 47.3%), whereas Duchemann *et al*.[19] recorded that the foremost frequent disease was sarcoidosis (n = 361, 42%). These variations may well be attributed to the small sample size in our study compared with the massive sample size within the others.

BRAF mutations are well represented in nonsmall cell lung cancer (NSCLC) for many years.[20] Changes in BRAF constitutively stimulate the MAPK pathway, creating steady upgrades bringing about cell development and multiplication, and protection from negative modulatory feedback signals.[21]

Several studies stated that BRAF mutation had a role in the pathogenesis of NSCLC. Brustugun *et al*.[22] unconcealed that the frequency of BRAF mutation was present in 2.3% among 646 cases of adenocarcinomas (ADCs) and analysis of BRAF mutation is one among the necessary steps of nonsquamous NSCLC subtyping, whereas different research found that BRAF mutation was found in 9%, 1.5%–3.5%, 2.8%, and 9.3% of NSCLC in Caucasian, European, Chinese and Iraqi patients, respectively.[13,23-25]

In 2017 BRAF targeted therapy, dabrafenib and trametinib are approved by FDA as a treatment modality of BRAFV600E mutant NSCLC.[26]

Kim *et al*.'s[27] study in South Korea reported the flexibility of targeting BRAFv600E mutation by using dabrafenib and trametinib drugs to treat patients with advanced ADC harboring BRAFv600E mutation, whereas not epidermal growth factor receptor/anaplastic large-cell lymphoma kinase aberration. Thus, these evidences pay the attention of researchers to analyze the genetic disruption of ILD cases with lung cancer.

A retrospective study in Japan (regarding genetic features of UIP-associated ADC) unconcealed that the proportion of $BRAF^{(v600E)}$ mutation in UIP-ADC and ADC without UIP was 2.5% and 0.5%, respectively, and KRAS mutation represent the foremost frequent and vital gene mutation in UIP-ADC (40%).[28]

In 2017, Hwang *et al*.[29] in their study regarding the genomic profile of lung cancer related to IPF found that BRAF mutation was vital in LC-IPF, and its proportion was 17.1%.

The above two studies showed a relevant relationship between BRAF mutation and carcinoma related to IPF, these findings may support our results of being all the ILD cases with mutant BRAF gene were of UIP (a subtype of IPF), so it may be taken it seriously as an early step of carcinogenesis in IPF patients.

BRAF mutation was present in 3.25% of lung cancer related to ILD of pneumoconiosis, drug-induced, and connective tissue disease-associated ILD subtypes as a part of French genetic study of pulmonary fibrosis with pulmonary tumors,[30] and it had been worth mentioning that BRAF mutations during this study were of non-V600E sort, and thus, as a result, our study was investigated BRAF(V600E) mutation alone. We must always do genetic tests relating to another sort of BRAF gene mutation in our patients particularly people who had no $BRAF^{V600E}$ mutation to make sure that there was a complete absence of BRAF mutations within the fibrotic lung diseases apart from IPF in this study.

During this study, there are some points of limitations that include the little sample size and lack of follow-up for those patients with positive results.

Conclusion

From the above discussion, we tend to conclude that BRAF V600E mutation may well be detected in ILD, particularly UIP. Workup is required to observe the prognosis of such patients with mutated BRAF gene, advanced study can be recommended in patients with ILP particularly UIP to exclude the BRAF V600E mutation in such patients because such mutation could be a risk issue for the long-term development of lung carcinoma.

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Conflicts of interest

There are no conflicts of interest.

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