Risk of cognition alteration and emotional frailty via circulating transcriptome in treatment naïve head and neck squamous cell cancer patients

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ABSTRACT

Background: There is a dearth of research examining the association between differential expression (DE) of genetic transcriptome associated with cognition alteration (CA) and emotional frailty (EF) in treatment naïve head and neck squamous cell carcinoma (HNSCC) patients. The present study was undertaken to identify the DE of mRNA of CA-EF in HNSCC tumor and correlate with clinical and other known genetic factors that promote oncogenesis as well as CA-EF.

Material and methods: Using Genome-Wide Association Studies, putative genes associated with CA-EF (P < 0.10) were identified. The DE of the mRNA of the thus selected genes were obtained from The CANCER GENOME ATLAS – HNSCC patients along with clinical details. The DE of mRNA pertaining to known factors such as inflammation, serotonergic and dopaminergic functions as well as clinical parameters were studied for association with the risk of DE of CA-EF. Appropriate statistics were performed and $P \leq 0.05$ was taken as significant.

Results: A total of 520 HNSCC patients formed study group. There were 77 (14.81%) patients at risk for CD, 41 (7.9%) for CI and 113 (21.73%) for EF risk. In all, 103 (19.81%) HNSCC patients of this cohort had DE of mRNA of genes associated with CA. Inflammation, circadian genes, mTOR pathway, invasion and metastasis set of genes had a significant association with the risk of DE of CA-EF.

Discussion: Transcriptome's have been postulated to mediate CA-EF by targeted action on human brain. Differential Expression of putative genes associated with CA-EF have been demonstrated in HNSCC tumor. These DE could predispose the patients to CA-EF by the action of gene-environmental as well as psycho-social constructs. As CA-EF could adversely influence the treatment and alter the quality of life among survivors, screening for CA-EF at HNSCC presentation becomes imperative.

1. Background

Head and Neck Squamous Cell Carcinoma (HNSCC) is one of the commonest malignancy among tobacco users with high incidence in South Asia. The clinical presentation of HNSCC are heterogenic and survival rates depend on several parameters. A large number of HNSCC are associated with use of tobacco and or alcohol.^{1,2} Chronic use of such substances may often be accompanied by un (under) diagnosed psychiatric co-morbidities.³

Treatment of cancer is reported to be commonly associated with cognitive alterations^{4–18} (CA, including cognitive impairment—CI— that refers to those secondary changes, denoting a reduction in an individual's mental abilities including but mostly limiting to perception, attention, language, action planning, learning and memory, thinking, reasoning, understanding, and problem solving; Cognitive disorders, CD refers to a set of disorders that significantly impairs the cognitive function of an individual to the point where normal functioning in society is impossible without treatment) and emotional frailty (EF).^{19,20}

There are epidemiological, longitudinal and biomarker based studies that reports of the effect of the radiological, surgical or chemotherapeutic effects on CA/EF among cancer survivors, notably from breast cancer. As such CA are common during or after chemotherapy, they are popularly referred in cancer literature as “Chemobrain” or “Chemofog”.^{4–18} Certain cancer longitudinal studies that included baseline assessments for CA revealed a high prevalence of neurocognitive dysfunction, in treatment naïve states. Prevalence of such CA during, post-treatment and survival period among HNSCC patients to extent of 47% of the study population.^{21–25} In a study, more than a fourth of the treatment naïve HNSCC patients showed decreased in executive function, processing speed, verbal learning, and verbal memory domains as also changes in brain structures as observed by brain imaging studies. These subsets of patients were reported to have difficulty in planning regular life, sequencing their daily activities and with multitasking.²² The prognostic value of CA/EF, particularly for elderly HNSCC patients, lies in the ability of participating in clinical decision making that could directly affect their quality of life.^{21,26} However, only few studies have

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investigated the effect of cancer on CA and EF, before or at diagnosis.^{22–24}

Identification of CA-EF in treatment naïve HNSCC patients is important because it may be associated with patient's i. Difficulty in understanding, imbibing information about the diagnosis and treatment of their disease, when CA are moderate/severe. ii. Difficulty in assessing the extent of the need of the physical and psychological care during treatment and post-treatment period. iii. Difficulty with consent process. iv. Assessing the risk of further decrease in CA during treatment due to acute and late toxicities. v. Potential difficulties in perceiving, reporting or monitoring changes in symptoms, which would lead to increased complications, decreased quality of life and possibly, increased physical and psychological distress.^{22–24}

The association of genes with CA and EF has been long studied.^{27–29} The Genome-Wide Association Studies (GWAS) have identified the association of several genes with these traits and been published.³⁰ These studies gave a unique opportunity to correlate the putative association of genes to traits such as CA (cognitive disorders/impairments) and EF. Alterations in tumors-gene expression via humoral and neural pathway could possibly reach the brain to cause CA-EF traits.¹² The extent of the differential expression (DE) of these putative genes of traits (CA/EF) in HNSCC population has not been reported earlier. These DE of genes could place the HNSCC patient at risk for development/expression of the CA/EF traits, notably when further modulated by Gene-Environment interactions.³¹ Assessment of CA-EF at risk population, via the GWAS and whole genome sequencing of tumor studies could help to assess the gene level changes and interplays, resulting in CA/EF that possibly has a direct bearing on the HNSCC treatment.

We attempt to study the DE of mRNA of CA/EF associated genes in a large set of HNSCC patients. We hypothesize that 1. a subset of HNSCC patients have DE of mRNA expression of CA-EF related genes; 2. Factors such as inflammation, HPA axis suppression, ribosomal protein alteration, serotonergic, noradrenergic, dopaminergic set of genes influences such DE of mRNA of CA-EF related genes; 3. Clinical parameters of HNSCC patients have no association with the DE of mRNA expression of the CA-EF genes.

2. Materials and methods

The sample population data for this study was collected from the cBioCancer Genomics Portal (<http://cbiportal.org>) using HUGO gene symbols.^{32–34} The portal is an open-access, depersonalized data resource with options for interactive exploration of multidimensional cancer genomics data sets. It currently provides access to combined data from more than 60,000 tumor samples from 225 cancer studies. Details of primary, treatment naïve, HNSCC were collected from this dataset. The clinical details and the mRNA expression z-score data from HNSCC reported from the Cancer Genome Atlas (TCGA) were included for this study.³⁴ For any particular gene considered, the mRNA z-Scores, obtained by processing the RNA Seq V2 RSEM (a software package for estimating gene and isoform expression levels from RNA-Seq data) at a higher set threshold (± 3 for predictors and ± 5 for outcome variables) for this study, as against the norm ± 2 to increase the quality of the gene-risk association. This portal employs the complex data processing algorithm to produce the z-score results, as expressed (above/down) of the set threshold for the expression of mRNA. All data were collected in the third week of September 2018. As the Z-score of mRNA expression is a measure of the deviation, only its association with possible factors are analyzed by this study. The results shall be interpreted with the fact that the impact of the differential expression (DE) would also depend on the contribution of the gene in the various functional pathways, environmental interaction, mutation of the upstream/downstream genes etc.,. Hence this present study would limit only to association of one or more genes expressing altered mRNA in HNSCC with the regards to risk of CA-EF.

TCGA definition of patients, clinical details of gender, cancer

staging, clinical staging, histology grading, Human Papilloma Virus (HPV) status, patient vital status and disease status (at the last presentation), smoking categories, mutation count, age at diagnosis and aneuploidy score, were employed in this study.

2.1. Predictor variables in the study

The common genes involved with the regulation of ribosomal protein synthesis and cell growth, PI3k-AKT-mTOR signaling pathway and those with invasion/metastasis as reported in cBioCancer Genomics Portal was collated and their mRNA expression, (Z-score ± 3) was dichotomized as DE or non-DE as from the website (<http://cbiportal.org>).^{32–34} Differential expression of set of genes from the inflammation mediated by chemokine and cytokine signaling pathway, Adrenalin and Nor-adrenalin biosynthesis were collated and classified as DE or non-DE.^{35,36} The serotonergic (concerned with serotonin synthesis, transportation, receptors - TPH1, TPH2, SLC6A4, HTR1A, HTR2A, HTR1B, HTR2C and MAOA) set of genes was obtained by manual search from literature. From Gene Ontology portal (<http://geneontology.org>), known human genes associated with Dopaminergic function, circadian genes (involved in sleep/wake cycle process; GO:0022410) and genes involved with chronic inflammatory response (GO:0002544) was collected. The DE of mRNA in the HNSCC samples was collected and categorized as DE or Non-DE. When one gene of the gene sets in the predictor variables was DE, it was considered to be DE.

2.2. Outcome variable

The Genome-Wide Association Studies were scanned using the previously curated, published database from Hong Kong (<http://jjwanglab.org/gwasdb>) was used to identify the putative causal association genes for the traits of cognitive disorders (CD), cognitive impairment (CI) and abnormal emotions (EF) were collected.³⁰ Only those genes that had the Prix Fixe score of ≥ 0.10 were included for the purpose of this study. The web portal employs Prix Fixe network to assess the shared-function or co-function networks to prioritize candidate causal gene among functionally related genes to draw a score, called Prix Fixe Score, depending on the gene's contribution to the expression of the trait.³⁰

The expression of such collected genes mRNA at a Z-score threshold of ± 5 , (to increase the quality of findings and minimize noises during estimation) was obtained from the www.cbiportal.com for the study population. The original gene expression values of the HNSCC tumor from study population were retrieved using the <https://xenabrowser.net/heatmap/> for studying the descriptive statistics.³⁷ The total number of DE genes for each patients for CI, CD and EF were calculated. For the trait expression of CA (CI and CD), when 2 or more genes had DE, the HNSCC subject was assumed to be at risk for CA (CI and CD). When a single gene of EF is DE, then it was assumed to be at risk for EF. This was based on the 90th percentile of the total number of genes DE in each of the set of genes.

2.3. Statistical analysis

The data thus collected were entered and analyzed using the Statistical Package for Social Services (SPSS, Version 23, IBM, IL, USA). The predictor variables were compared with the outcome variable using chi-square test, appropriate statistics of one-way of ANOVA and Kruskal-Wallis test as required. $P \leq 0.05$ was taken as significant.

3. Results

The mRNA expression and DE data of 520 HNSCC patients were collected for this study. The relevant clinical details were collected. The distribution of genes involved with CI, CD and EF are depicted in Figs. 1 to 3. In all there were 45, 67 and 50 genes (Prix Fixe score ≥ 0.10)

Fig. 1. Schematic infographic showing genes associated with cognitive disorder in different human chromosomes.

associated with CI, CD and EF respectively, in 158 (30.38%), 196 (37.7%), 113 (21.6%) number of HNSCC patients in the study population. The scores of the genes, mean $\pm$ standard deviation, median expression along with Prixe Fixie score are given in Supplementary table for CI, CD and EF.

There were 77 (14.81%) patients at risk for CD, 41 (7.9%) for CI and 113 (21.73%) for EF risk. In all, 103 (19.81%) HNSCC patients of this cohort had abnormal DE of mRNA of genes associated with CA. The inflammation Mediated by Chemokine and Cytokine Signaling Pathway was involved in 460(88.5%), 100($n = 19.23\%$) with Ribosomal protein synthesis and cell growth Protein set of genes involvement, 209 (40.2%) patients with PI3k-AKT-mTOR Signaling Pathway set of genes involved, 188 (36.15%) of the HNSCC Metastasis and Invasion set of genes had DE. With the others, Serotonergic set of genes ($n = 78$, 15%), Adrenalin/Nor-Adrenergic group of genes ($n = 178$, 34.23%), Dopaminergic set of genes ($n = 133$, 25.58%), circadian Genes ($n = 81$, 15.58%) and Chronic inflammation set of genes ($n = 277$, 53.27%) were involved (Tables 1 and 2).

On comparing the CI and CD, it was observed that of the HNSCC patients who are not risk for CI ($n = 479$, 92.1%), 417 (94.1%) had no risk for CD while 62 (80.5%) were at risk for CD. While, among those patient at risk for CI, 26 (5.9%) were not at risk for CD while 15

(19.5%) was at risk for CD. This difference was statistically significant. ($P = 0.000257$). The association of CI and CD with EF was not statistically significant. ($P = 0.077$ and 0.06, respectively).

The difference between gender ($P = 0.028$) with a male preponderance and cancer staging ($P = 0.006$) was significant for CD. The CD was significantly associated with PI3k-AKT-mTOR Signaling Pathway ($P = 0.004$), Metastasis and Invasion set of genes ($n = 0.001$), Serotonergic set of genes ($P = 0.015$), Adrenalin/Nor-Adrenergic group of genes ($n = 0.015$) and Dopaminergic set of genes ($P = 0.000$). (Tables 1,2,3).

Risk of CI was significantly correlated with smoking ($P = 0.005$), HPV status ($P = 0.05$), histology grading ($p = 0.006$) and patient status ($P = 0.029$). The CIs were Inflammation Mediated by Chemokine and Cytokine Signaling Pathway ($P = 0.012$), Ribosomal protein synthesis and cell growth Protein set of genes ($P = 0.041$), PI3k-AKT-mTOR Signaling Pathway ($P = 0.001$), Metastasis and Invasion set of genes ($P = 0.026$), Serotonergic set of genes ($P = 0.000$), Adrenalin/Nor-Adrenergic group of genes ($n = 0.000$), Dopaminergic set of genes ($P = 0.000$), Circadian Genes ($P = 0.000$) and Chronic inflammation set of genes ($P = 0.031$). Similarly for EF, smoking ($P = 0.01$), clinical staging ($P = 0.004$) and cancer staging ($P = 0.039$) were associated with statistical significance (Tables 1–5).

Fig. 2. Schematic infographic showing genes associated with cognitive impairment in different human chromosomes.

4. Discussion

The onset of CA and EF among cancer patients, in the treatment and post-treatment phase has been adequately documented.^{21–25} There are several hypothesis being proposed to explain this phenomenon.¹² The impact of the CA and EF on the treatment has been discussed earlier.^{21–25} Notably, such changes have been often noted with changes in the brain, as identified by the brain imaging studies.²⁵ The International Cognition and Cancer Task Force in the past decade has listed several recommendations in areas of such CA.⁴

The occurrence of CA at the time of clinical presentation had been an issue of contention. Studies among HNSCC patients have shown different results. In the recent past, a HNSCC study that show no CA at time of presentation in a case-control setting²³ while certain other study demonstrated about 50% of the study population presenting with at least mild CA.^{22,24}

The available literature supports a notion that CA occurs in a group of vulnerable, subsample of cancer patients, even prior to treatment.^{12,23,24} The CA mostly involved total/delayed recall on verbal learning tasks, manual dexterity, executive functioning tasks, visual scanning tasks, verbal fluency, processing speed and auditory attention tasks as compared to normative samples.²² The factors that promote the

traits are not fully elucidated. The mechanism by which the tumor transcriptome (the sum total of all the messenger RNA molecules expressed from the genes of an organism) exert an influence on brain has been postulated through animal and human models.¹² The role of inflammatory mechanisms and behavior, particularly the slow-priming effect of tumor on immune system, alterations in sleep dysregulation (circadian rhythm changes) are some of the several mechanism that has been suggested by which the tumor transcriptome influences the brain.^{12–18}

Appearance of CA-EF symptoms in the preceding months of cancer diagnosis has often been overlooked in oncological setting, as most of these symptoms are non-specific and also are reported by a substantial section of the non-cancer population. Often in pre-diagnostic situation, routine screening for depression-anxiety, CA-EF are often overlooked, hence there is a paucity of data on the pre-existence of CA-EF in cancer and HNSCC particularly.^{22,24} This assumes prognostic importance because, in breast cancer, a recent neuroimaging study identified that existence of pre-treatment dysfunction in executive function neural networks was more strongly associated with post-treatment fatigue and CA.²⁴

The genetic mechanism underpinning of emotions, CI and CD has been previously reported.^{20,27–29} The GWAS studies have served as an

Fig. 3. Schematic infographic showing genes associated with emotional frailty in different human chromosomes.

excellent tool to associate the genes to the traits. The collation attempt of published GWAS studies, as employed in this study, has been previously tested and published.³⁰ The online portal facilitates collation of several studies to identify and prioritize genes of interest. Only putative genes associated with the traits (CI, CD, EF) with a score ≥ 0.1 was employed for this study.³⁰ Furthermore, Z-score of mRNA expression was set at ± 5 , instead of 2 to identify high risk patients only. These measures would hence identify the most DE and highly relevant genes. In addition, for the predictor variables, this study utilizes a Z-score of ± 3 , instead of 2 to possibly include only those genes that have a significant DE as compared to the others in the study population.

This study attempted to study the association of the DE of putative genes of CI, CD, EF in HNSCC patients and correlate the same with genetic markers of inflammation, cancer progression, Adrenalin/Nor-Adrenergic expression, Dopaminergic, circadian pattern and clinical details. In all 103 (19.81%) HNSCC patients had exhibited at risk for CA and 95 (18.27%) for EF. This indicates that about 1 in 5 HNSCC patients have a DE of genes that concerns risk associated with CA and EF. Clinical studies quote of 1 in 2 patients having at least mild CA. The difference could stem from genetic association and the gene-environment interaction.^{21,31} Trait expression is dependent on several factors including the Gene-environment interaction that is not covered in the

scope of this study. This could have contributed to the higher clinical manifestation. This finding also could stem from the stringent, high quality of inclusion criteria set (Prix Fixie Score ≥ 0.1 ; ± 5 Z-score of mRNA). To the best of our knowledge, there is no reported study that compares the DE gene expressions of CA-EF with clinical parameters of HNSCC. Hence the results of the study cannot be compared with existing literature.

The present study reveals that males are at higher risk for CD ($P = 0.028$) which has been observed in previous studies.^{21–25} Smoking tobacco appears to influence the CI and EF with statistical significance. The relationship between tobacco use and CA has been previously documented and the present study is in concurrence with the same.²³ Clinical staging of HNSCC has statistical significant association with EF, indicating a strong association with progression of disease and there is no reported study in literature comparing EF reported till date to confirm or refute the present findings.

HPV status difference is statistically significant for CI ($P = 0.05$). It is unlikely the HPV status, per se relates directly to CA. But the fact that significant association could be due to the fact that patients with HPV may have less smoking history and alcohol use, fewer comorbidities and thus have substantially less risk.²³

Clinical staging is associated with statistical significance with EF

Table 1

Association of risk of genetic cognition and emotional alterations among various clinical parameters in the study group.

Category	Sub-groups	Risk of cognitive				At risk for emotional alterations	
		Disorder at risk		Impairment at risk		(n = 113) n (%)	P-value
		(n = 77) n (%)	P-Value	(n = 41) n (%)	P-Value		
Gender	Female (n = 136)	13 (16.9)	0.028	10 (24.4)	0.477	25 (22.1)	0.163
	Male (n = 384)	64 (83.1)		31 (75.6)		88 (77.9)	
Smoking	Non-smoker (n = 117)	17 (22.7)	0.686	9 (23.1)	0.005	20 (17.9)	0.010
	Current smoker (n = 177)	22 (29.3)		9 (23.1)		49 (43.8)	
	Reformed, > 15 years (n = 73)	11 (14.7)		1 (2.6)		7 (6.3)	
	Reformed, ≤ 15 years (n = 139)	25 (33.3)		20 (51.3)		36 (32.1)	
	Reformed, unknown (n = 2)	–		–		–	
HPV Status	Negative (n = 243)	33 (86.8)	0.565	21 (75)	0.050782	67 (89.3)	0.553
	Positive (n = 36)	5 (13.2)		7 (25)		8 (10.7)	
Clinical staging	Stage I (n = 20)	1 (1.3)	0.5	1 (2.5)	0.576	2 (1.8)	0.004
	Stage II (n = 98)	10 (13.2)		11 (27.5)		11 (9.8)	
	Stage III (n = 105)	17 (22.4)		5 (12.5)		18 (16.1)	
	Stage IVA (n = 265)	45 (59.2)		22 (55)		75 (67)	
	Stage IV B (n = 11)	2 (2.6)		1 (2.5)		4 (3.6)	
	Stage IV C (n = 7)	1 (1.3)		–		2 (1.8)	
	Stage I (n = 27)	–		2 (5.7)		3 (3.2)	
Cancer staging	Stage II (n = 74)	5 (7.8)	0.006	6 (17.1)	0.944	7 (7.4)	0.039
	Stage III (n = 105)	11 (17.2)		6 (17.1)		16 (16.8)	
	Stage IVA (n = 254)	45 (70.3)		21 (60)		66 (69.5)	
	Stage IVB (n = 12)	2 (3.1)		–		3 (3.2)	
	Stage IV C (n = 1)	1 (1.6)		–		–	
	Grade 1 (n = 62)	5 (6.7)		–		9 (8.2)	
	Grade 2 (n = 301)	40 (53.3)		20 (52.6)		71 (64.5)	
Histology grading	Grade 3 (n = 123)	24 (32)	0.117	14 (36.8)	0.006	26 (23.6)	0.343
	Grade 4 (n = 7)	1 (1.3)		–		–	
	Grade X (n = 18)	5 (6.7)		4 (10.5)		4 (3.6)	
	Dead, unknown (n = 127)	20 (26)		12 (29.3)		18 (15.9)	
	Alive, disease free (n = 249)	35 (45.5)		11 (26.8)		51 (45.1)	
Patient, Disease status	Alive, Disease recurred/progressed (n = 50)	10 (13)	0.664	7 (17.1)	0.029	14 (12.4)	0.10
	Dead, Disease recurred/progressed (n = 94)	12 (15.6)		11 (26.8)		30 (26.5)	

alone while the Cancer code staging was significantly associated with CD and EF. This indicates that as the clinical progression of HNSCC process proceeds (from Stage I to IV), the risk of EF also increases. This could be related to the fatigue, depression, stress and possibility of the direct effect of the aggressiveness of the disease. However, as there is no previous study among HNSCC patients in this regard, the findings cannot be compared. Histological grading revealed that CI was at higher risk in Grade 2 lesions, indicating the fact that the slow priming effect of tumor has more significant role in CA.^{12,13} Survival status has significant status only with CI and we could not identify any plausible reason for this finding.

CI was observed with cancer related PI3k-AKT-mTOR Signaling Pathway and metastasis and Invasion set of genes with statistically significant association. Similarly, the CI was strongly associated with serotonergic, Adrenalin/Nor-Adrenergic and dopaminergic genes.

These set of genes are commonly associated with depression, suicidal thoughts and have been known to be hyper-expressed in HNSCC patients with clinical evidence of depression and suicidal tendency.^{3,38,39} This strong association points out the possible existence of transcriptome/gene-based predilection of depression and CA in a subset of HNSCC patients.

CD was associated with statistical significance with the DE expression of genes concerned with inflammation, circadian rhythm, ribosomal proteins, cancer pathways, Adrenalin/Nor-Adrenergic and dopaminergic genes. Abnormalities of ribosomes have been associated with early Alzheimer's and the significant association of CD with DE of mRNA of genes associated with Ribosomal protein synthesis and cell growth Protein indicate the common nature of the cognitive deficits in both the conditions.⁴⁰ Inflammation, Circadian rhythm and sleep disturbances are reported to be directly associated with clinical

Table 2

Association of risk of genetic cognition and emotional alterations between various pathways, sets of genes and databases considered.

Involved Differential Expression of mRNA from genes by/of	Risk of Cognition				At risk for emotional alterations	
	Impairment		Disorder		(n = 113) n (%)	P-value
	At Risk (n = 41) n (%)	P-value	(n = 77) n (%)	P-Value		
Inflammation Mediated by Chemokine and Cytokine Signaling Pathway (n = 460)	39 (95.1)	0.123	74 (96.1)	0.012	102 (90.3)	0.311
Ribosomal protein synthesis and cell growth Protein set of genes (n = 100)	7 (17.1)	0.451	21 (27.3)	0.041	28 (24.8)	0.062
PI3k-AKT-mTOR Signaling Pathway (n = 209)	25 (61)	0.004	44 (57.1)	0.001	56 (49.6)	0.015
Metastasis and Invasion set of genes (n = 188)	22 (53.7)	0.013	36 (46.8)	0.026	39 (34.5)	0.384
Serotonergic set of genes (n = 78)	14 (34.1)	0.001	16 (20.8)	0.089	15 (13.3)	0.339
Adrenalin/Nor-Adrenergic group of genes (n = 178)	21 (51.2)	0.015	42 (54.5)	0.000	41 (36.3)	0.340
Dopaminergic set of genes (n = 133)	21 (51.2)	0.000	40 (51.9)	0.000	31 (27.4)	0.345
Circadian Genes (n = 81)	9 (22)	0.170	24 (31.2)	0.0000	23 (20.4)	0.078
Chronic inflammation set of genes (n = 277)	26 (63.4)	0.117	49 (63.9)	0.031	67 (59.3)	0.089

Table 3
Genetic Risk of Cognitive Disorder correlated with HNSCC parameters.

	Cognitive Disorder Genetic risk	Mean	Std. Deviation	95% CI for Mean		Minimum	Maximum	P– value
				Lower	Upper			
Mutation Count	Absent	146.78	214.83	126.72	166.84	0.00	3181.00	0.415
	Present	151.87	202.91	105.82	197.92	0.00	1668.00	
Diagnosis Age	Absent	61.04	11.97	59.92	62.16	19.00	90.00	0.436
	Present	59.90	11.31	57.33	62.46	26.00	85.00	
Aneuploidy Score	Absent	11.16	7.20	10.48	11.85	0.00	32.00	0.104
	Present	12.63	7.12	10.99	14.26	0.00	30.00	
Person Cigarette Smoking History Pack Year Value	Absent	46.28	36.68	41.72	50.84	0.02	300.00	0.605
	Present	43.31	26.52	35.35	51.28	0.50	120.00	

Table 4
Genetic Risk of Cognitive Impairment correlated with HNSCC parameters.

	Cognitive Impairment – Genetic risk	Mean	Std. Deviation	95% CI Mean		Minimum	Maximum	P-value
				Lower	Upper			
Mutation Count	Absent	145.91	217.44	126.39	165.43	0.00	3181.00	0.189
	Present	166.56	151.08	118.88	214.25	19.00	742.00	
Diagnosis Age	Absent	60.88	11.84	59.81	61.94	19.00	90.00	0.970
	Present	60.80	12.42	56.89	64.72	35.00	83.00	
Aneuploidy Score	Absent	11.25	7.19	10.60	11.91	0.00	32.00	0.163
	Present	12.97	7.24	10.56	15.39	1.00	25.00	
Person Cigarette Smoking History Pack Year Value	Absent	45.96	36.01	41.65	50.26	0.50	300.00	0.0835
	Present	44.42	27.00	33.28	55.56	0.02	110.00	

Table 5
Genetic Risk of Emotional Frailty correlated with HNSCC parameters.

	At risk for emotional alterations	Mean	Std. Deviation	95% CI for Mean		Minimum	Maximum	P-Value
				Lower	Upper			
Mutation Count	Not involved	147.72	219.08	126.37	169.07	0.00	3181.00	0.811
	At high risk	146.87	189.99	111.45	182.28	0.00	1668.00	
Diagnosis Age	Not involved	61.29	11.77	60.14	62.44	24.00	88.00	0.127
	At high risk	59.36	12.18	57.09	61.63	19.00	90.00	
Aneuploidy Score	Not involved	11.19	7.22	10.47	11.91	0.00	30.00	0.263
	At high risk	12.06	7.12	10.71	13.42	0.00	32.00	
Person Cigarette Smoking History Pack Year Value	Not involved	44.98	36.46	40.11	49.85	0.02	300.00	0.490
	At high risk	48.20	31.92	41.00	55.40	1.00	180.00	

depression, anxiety and suicidal tendency in HNSCC patients. Possibly, CA are also associated with same or similar pathways.^{3,12,18,38,39}

It is to be noted that the PI3k-AKT-mTOR Signaling Pathway, that is associated with many cancer and pain, is associated with statistical significance with CI, CD and EF. The persistent peripheral nociception-induced enhancement of synaptic plasticity in entorhinal cortex - hippocampal pathways of brain has been suggested. This is dependent on mTOR-S6K signaling pathway.^{41,42} The resultant behavioral effects of this nociception, includes signs of depression and anxiety. Possibly, the strong DE of genes associated with PI3k-AKT-mTOR Signaling Pathway, could mediate CI, CD and EF when the circulating transcriptome reaches the brain via the humoral and neural pathway.⁴¹

4.1. Strength of the present study

Cognitive Alteration affects the quality of life of HNSCC patients. Expression of at-risk genes for CA-EF may have a significant influence on the course of HNSCC treatment. Possible existence of correlation of CA-EF in tumor mRNA has been established by this study. To the best of our knowledge, this is the first study that attempted to correlate DE of mRNA of CA-EF to clinical details as well as other DE of genes that could influence the progression of HNSCC, using large cohort of HNSCC patients using public cancer genomic database. The study relies on highly validated, cross-access databases, large scale data mining

capacities of several online websites using the analytical powers. Furthermore, much valid Prix Fixie scores have been used to identify genes associated with CA-EF.

4.2. Limitations of the present study

The present study relies on pre-existing knowledge about CA-EF, which has been updated till mid-2015. Errors, if any during the sequencing, high background levels due to cross-hybridization, narrow dynamic range of detection due to higher background, signal saturation or both remains a possibility, though RNA-Sequencing are supposed to be gold standard. Differences in transcriptome assays, in detection of particular (protein coding), non-coding mRNAs, mRNA transcripts and protein outputs of a gene besides other circulating free genetic materials hampers the deduction of gene expression within and between tissues. Also, the studies only concentrates on the association and not the “tightness” of the genetic control of the neural process or dynamic interactions between various neural circuitries.

Being a secondary database analysis, it has the strength of large sample size (n = 520) but with less/absence of adequate clinical details including those pertaining to signs, symptoms and quantification of CA-EF. The other limitation is that the study associated only with tumor level mRNA expression and not in blood or brain, though previously hypothesized.¹² Hence the findings from the studies should be

interpreted with caution, till they are replicated in prospective or a well-designed, independent sample cohort, preferably in multi-centric and population settings. The phenotypic heterogeneity of both HNSCC and CA-EF would still remain a challenge.

4.3. Clinical implications

The survival rates among HNSCC survivors is increasing and the longevity of life comes with burden of CA-EF. Inherent GA in HNSCC could be a possible cause of CA-EF in these patients. Precautionary early screening would be useful in this subset of HNSCC patients. When more knowledge and clarity on CA-EF and the DE of CA-EF genes emerge, it would provide alternative solutions to minimize the CA-EF at the baseline, during and after treatment. With a different set of documentation and approach recommended for multimorbid conditions, CA-EF occurring in HNSCC patients would be needed to have a highly personalized approach such as Sharing Evidence Routine for a Person-Centred Plan for Action (SHERPA) framework.⁴³ In this regard, the neuro-psychiatric field leadership as well as stakeholders such as The International Cognition and Cancer Task Force need to come with specific recommendations, guidelines and protocols suitable for low resource settings to make the quality of life better for those HNSCC patients with CA-EF.

5. Conclusion

From above discussions, it could safely assumed that there is 1. A definite subset of HNSCC patients have DE of mRNA of known genes associated with CA-EF; 2. The known factors such as inflammation mediated by chemokine and cytokine signaling pathway, ribosomal protein synthesis and cell growth set of genes, PI3k-AKT-mTOR signaling pathway and those with invasion/metastasis associated genes as well as the nor-adrenergic group of genes is associated with the DE of CA-EF associated genes with statistical significance. As CA-EF are important for better quality of life of HNSCC patients, more studies would be needed to establish the risk of DE of CA-EF genes in HNSCC and transcriptome from a well-designed, multi-centric, longitudinal cohort with more detailed subjective and objective neurocognitive tests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jobcr.2019.03.001>.

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