Analysis of Mucosal- Associated Invariant T Cell Levels And Their Correlation With Tumor Immune Status In Patients With Brain Tumors

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Abstract: Introduction: Primary brain tumors refer to a heterogeneous group of tumors arising from cells within the CNS, and can be benign or malignant. In recent years, experimental evidence suggests that, despite the presence of blood-brain barrier (BBB) and lack of discrete lymphoid tissue, there are immunocompetent cells (TILs-Tumor infiltrating lymphocytes) within the brain parenchyma. Many of these cells are T-Lymphocytes known as CD8+ Mucosal-associated invariant T (MAIT) cells, which proliferate with stimulation, and are cytotoxic to tumor cells in vitro. To evaluate the frequency of unconventional MAIT cells in the setting of brain tumors by expression analysis of their three defining markers CD3D, KLRB1 (CD161) and a TCR a-chain variant Va7.2 (TRAV1-2 gene). **Methodology:** The study involved collecting EDTA blood samples and fresh brain tissue biopsies from selected population followed by RNA extraction and cDNA synthesis for measurement of relative expression analysis in blood and tissues samples. Expression data was generated by semi-quantitative real time PCR. **Results:** The outcome of this study demonstrated higher expression level of CD3D, KLRB1 (CD161) and a TCR a-chain variant Va7.2 (TRAV1-2 gene) in the blood of patients compared to the tissue samples showed little to no transmigration of these MAIT cells in the diseased tissues. Also, the expression of genes under-investigation was observed decreasing in both blood and tissue samples with the progression of tumor. **Conclusion:** MAIT cells might undergo depletion in the tumor micro-environment due to chronic activation induced exhaustion of these cells.

Keywords: Neurology, Brain Tumor, Mucosal Immunity, MAIT cells, TILs, Immune correlation, Expression Analysis

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Introduction

Globally primary and metastatic brain tumors establish the 3rd leading cause of cancer-related deaths [1].A great proportion of brain tumors in adults (86%)are gliomas, constituting a set of malignant brain tumors that incorporates highly aggressive gliomas/glioblastomas and less-aggressive gliomas as astrocytoma, oligodendroglioma, and unspecified gliomas [2]. Theimmunotherapeutic capability of T lymphocytes have concentrated on conventional CD8 T cells that perceive peptide-antigen (Ags) introduced by MHC class I particles. The

immunopathology by the commitment of CD4 T cell assist against MHC class-II restricted tumor peptide antigens (Ags) [3].

A populace of un-conventional T cells termed as 'MAIT cells (mucosal-associated invariant T-cells)' has evoked the interest of immunologists due to the lavishness of these cells in humans by introducing a specified MHC class I–like molecule 'MR1'. These MAIT cells utilize a diverse repertoire of T- cell receptors (TCRs) with common antigen specificity that are preserved over species [4].MAIT cells in human peripheral blood constitute 1-10% of all the T-cells. MAIT-cells manifest a semi-invariant and an evolutionary conserved TCR-α chain comprising a distinctive pattern of gene- rearrangement i.e. TRAV1-2- TRAJ33/12/20 (iVa7.2 joins with Ja-segment Ja33, Ja12, Ja20) in humans. MAIT cells' TCR-α chain couples with limited TCRβ repertoires. Majority of MAIT cells ~90% are CD8$^+$,manifesting either CD8ab or CD8aa and a minor CD8/CD4 DN (~7%) or CD4$^+$ (<1%)populations [5].

MAIT cells get activated via MR1 dependent or MR1 independent manner, a characteristic these cells share with other unconventional and innate-like t-cells as γδ T cells and iNKT (invariant natural-killer T) cells [6].Human MAIT -cell receptors identify MR-1 complex loaded with VB2 metabolite antigen and therefore directs the over-expression of markers-CD69, CD161, and CD25, release TH1-type cytokines (as TNF-α and IFN-γ) and TH17- type cytokines (IL-22 and IL-17). Activation of MAIT cells can also be mediated via cytokine signaling [IL-18 and IL-12] in an MR1 independent fashion and this phenomenon has been observed in various infectious and non-infectious inflammatory diseases [7]. In professional antigen-presenting cells (APC) toll-like receptor (TLR) signaling brings the expression of a variety of pro-inflammatory cytokines like IL-18 and IL-12, and thus TLR8, for these acts as a co-stimulatory signal in the activation of primary MAIT cells.

MAITS cells' vigorous reaction is mediated via the IL-18/IL-12 receptors signaling as MAIT cells equipped with countless cytotoxic-effector fragments such as granzymes and perforins [8]. Stimulated MAIT cell when co-cultured with HCT116 cells boosted IL-17 production and enforced HCR116 cell-cycle abolition at the G2/M stage in a dose-dependent and contact method and that was negated when treated with anti-MR1 [9]. It can be correlated that these unconventional CD8+ MAIT cells behave in the tumor-microenvironment as anti-tumor cytolytic effector cells with their role being interrupted in pro-tumor phenotype needs to be investigated. KLRB1 gene that encodes for CD161 is one of the most promising prognostic markers of MAIT cells in solid tumors. Therefore regarding the Tumor study investments, the role of increased levels of CD161, CD3, and a-chain variant Va7.2-Ja33 expressed by MAIT cells is yet to be established [10].

Material And Methods
Samples Collection, Transportation And Storage:
Sixty diagnosed cases of Brain Tumors with signed informed consent form by attendants or patients directly, were enrolled in the study along with 10 healthy controls. Out of 60 enrolled subjects, 40 have been screened out having complete medical history and biopsy tissue available. Special precautions were taken into consideration during the collection and transportation. Blood sample of patients was brought in EDTA vacutainer tubes and immediately stored in the in ice-box containing ice-packs. Brain biopsies were taken in sterilized cryo-vial and immediately snap-freeze in Liquid Nitrogen (LN) filled in stainless steel thermos. Samples stored according to standard protocols were then transported from the General Hospital, Neurosurgery Ward Lahore to the research laboratory of the School of Life Sciences department Forman Christian College (A Chartered University) Lahore and stored at -80°C temperature till further processing.

Inclusion Criteria: Patients with a diagnosed brain tumor of any type (meningioma-glioma) and grade (I-IV), Male and Female patients, aged between 18 and 70 years, Patients with a

histologically confirmed brain tumor, WHO performance status 0-2, with written informed consent and comply with the study protocol and procedures. No prior immunotherapy applied/ alkylating agents/ radiation to the brain and $1cm^3$ of available tissue size was included.

Exclusion Criteria: Prior treatment for brain tumors at study entry, Prior treatment with temozolomide, Use of immunosuppressant drugs except for steroids, Participants with other active malignancy in the past 3 years excluding in situ tumors.

RNA Extraction, Quality check and cDNA synthesis:
RNA was extracted using (Invitrogen TRIzol reagent: Catalog #15596026, USA) as per manufacturer's instructions for both samples types. Quality and quantity was checked by Nanodrop 2000/2000c spectrophotometer (Thermo Scientific). Only RNAs giving 260/280 and 260/230 ratio more than 1.5 along with depiction of clear bands on agarose gel electrophoresis were processed for cDNA synthesis. Otherwise re-precipitated or extracted again. cDNA was synthesized by using a Thermo Scientific kit (RevertAid First Strand cDNA Synthesis Kit: Catalog #K1622, USA) as per the manufacturer's protocol.

Primer Designing and Optimization
The primers were designed on a serial cloner by using the consensus CDS sequence of specific genes from the NCBI database and then primer specificity or universality was checked by primer-BLAST or BLASTn respectively. Primers were optimized using a gradient PCR thermocycler (Bio-Rad T100-Thermocycler, USA) to get the optimal annealing temperature. Their melting temperatures (Tm) and amplicon properties were optimized. The sequences of the designed primers (forward and reversed) are mentioned in Table 1:

Table 1: List of Primers

Primer		Primer Sequence
Va7.2 (TRAV1-2)	*Forward Primer*	5' F-CTACCTTCTGCAGACTCCAATG '3
	Reverse Primer	5' R-CAGAGGTAAGAGGCAGAGTC '3
CD161 (KLRB1)	*Forward Primer*	5' F-CCGGGTCTCTTAAACTGCC '3
	Reverse Primer	5' R-GAGCCGTTTATCCACTTCC '3
CD3D	*Forward Primer*	5' F-CAGCATCACATGGGTAGAGG '3
	Reverse Primer	5' R-CAGCAAAGCAGAAGACTCC '3

Expression Profiling by Agarose Gel Electrophoresis:
Agarose gel (1.2%) was prepared using 1.2g of agarose and dissolved in 100ml of 1X TAE buffer. DNA ladder of 50bp was used to access the approximate size of the genes. Safe green was used as a loading dye and the gel was run for 50-55 minutes at 85V; allowing the ladder to open fully. Gene profiles were then visualized under UV light in the GEL-DOC.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)
RT-qPCR was carried out using Bio-Rad's CFX 96 qPCR system to analyze the expression of mRNA HIF-1α, VEGF-A, ANG-2, FGF-2 and uPA. It was done by using (ThermoFisher Scientific SYBR Green qPCR Master Mix: Catalog# 4309155, USA) as instructed by the manufacturer.

Statistical Analysis of Results:
The results are analyzed by using densitometry with the help of NIH Image J software (1.52a version). The software helps to calculate the band intensity, its area and pixels. Band intensity of

each sample PCR product; reference gene (β-ACTIN) as well as our specific genes for analysis; has been calculated. The band intensity values are calculated and noted. The ratio between the values of our specific genes and β-ACTIN was calculated for the quantitative analysis of gene expression. Further comparative analysis plots for our specific genes were recruited from Microsoft Excel (version 2013). These comparison analysis plots helped us draw more valuable conclusion about our data.

Results
Demographical Data
Out of 60 patients enrolled in the study, 40 patients with brain tumor were selected for analysis having complete medical history and biopsies available. Patients were categorized into groups based on the type of tumor they are suffering from as shown in **Table 2**. Group 1 Meningioma (n=10), Group 2 Medulloblastoma (n=4), Group 3 Pituitary adenoma and craniopharyngioma (n=7), Group 4 Glioma (n=6), Group 5 Glioblastoma (n=8) and Group 6 Astrocytoma (n=5).

Table 2: Demographic Profile of Brain Tumor Patients

Study Groups	Brain Tumor Types	Gender	Mean Age $\pm$SD
Group 1	Meningioma (n=10)	Male (n=3)	40 ± 3
		Female (n=7)	45 ± 2
Group 2	Medulloblastoma (n=4)	Male (n=3)	22 ± 1
		Female (n=1)	18
Group 3	Pituitary adenoma and craniopharyngioma (n=7)	Male (n=5)	26 ± 2
		Female (n=2)	24 ± 1
Group 4	Glioma (n=6)	Male (n=3)	21 ± 3
		Female (n=3)	29 ± 2
Group 5	Glioblastoma (n=8)	Male (n=8)	40 ± 8
Group 6	Astrocytoma (n=5)	Male (n=2)	23 ± 7
		Female (n=3)	36 ± 9

Clinical Characteristics of Subjects
The patients with meningioma presented with history of headache which became worsen at morning, blurred vision, memory issues and episodes of fits. The patient with medulloblastoma presented with symptoms of headache, nausea and vomiting, difficulty in walking, tiredness, weakness and visual disturbances. Pilocytic astrocytoma patient had a history seizures, personality changing issues, headache worsen on awaking in morning, nausea and, vomiting. Craniopharyngioma patient had past history of headache, balance problems, frequent urination, visual disturbances, confusion and mood swings. The patients with pituitary adenoma had marked visual disturbances (diplopia then vision loss), headache, nausea and vomiting, symptoms of anxiety and depression along with behavioral changes. Glioblastoma patients had history of frequent headache, urinary incontinence, memory and visual issues. The patients with glioma experienced symptoms of headache, irritability, urinary incontinence, memory and visual disturbances. Blood profiles of patients is shown in **Table 3.**

Table 3: Blood Profile of Brain Tumor Patients

Complete Blood Counts				
	Hb (11.5-16.9g/dl)		WBC (4-11X10^9/L)	
	Mean ($\bar{x}$)	Standard Deviation (S)/σ	Mean ($\bar{x}$)	Standard Deviation (S)/σ

| Males	10.6	±1.1	11.2	±1.4
Females	10.4	±1.2	11.12	±1.3

Semi-Quantitative Rt Pcr Analysis Of Specific Genes

Mucosal Associated Invariant T cells are defined by the presence of three surface markers *CD3D, KLRB1* and *TRAV1-2*. The relative expression of these genes in all blood and tissues samples was observed on gel-electrophoresis (1.2% agarose) and by graphical data.

Expression analysis of *CD3D*: Higher expression of *CD3D* was observed in the blood samples of all tumor patients. And in the tissue samples of patients, significant expression was observed only in patients with histologically least-aggressive tumors (Meningioma) and in a glioblastoma (of IDH mild type) sample**shown in Figure 1 (A and B).**
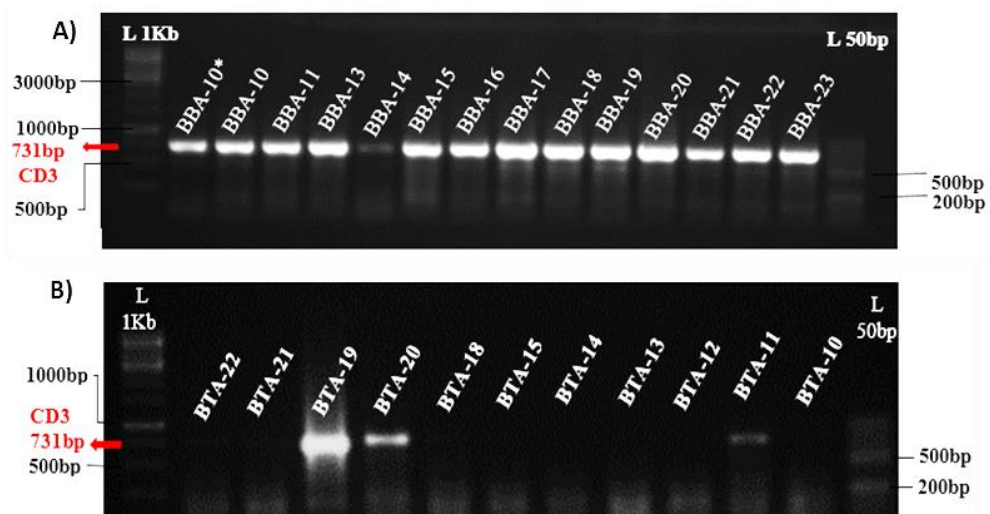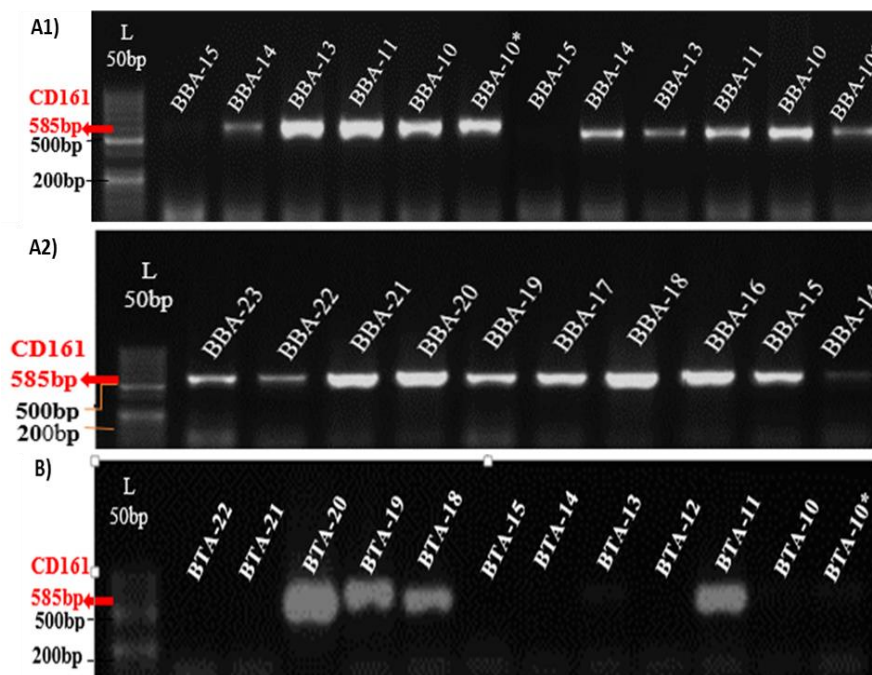

Figure 1: Gene expression of *CD3D*.Visualization of 731bp product CD3D gene on 1.2% agarose gel. **(A)** Expression profile on blood samples. **(B)** Expression on tissue samples. Samples IDs: (**BA*** Blood Samples analysis**)** (**TA***Tissue Samples analysis**)**

The relative expression of *CD3D* in blood was higher as compared to the biopsy samples except for 2 meningioma cases showing that expression of gene was found higher in tumor tissue as shown in Figure 2. The expression of *CD3D* was observed decreasing as the malignancy of tumor increases. Agarose gel showing PCR amplification of *CD3D* and graphical representation of *CD3D* expression by densitometry**.**
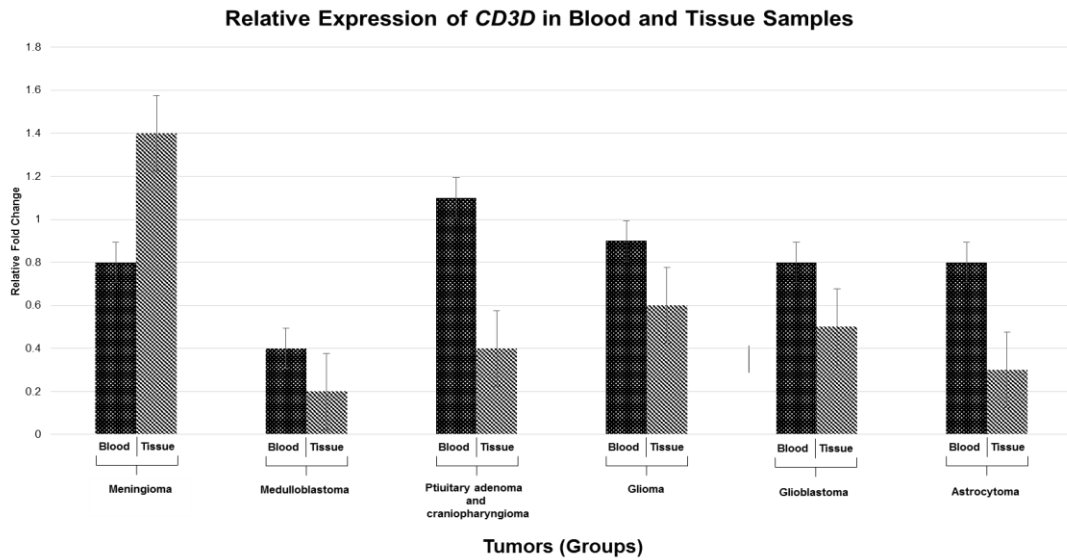
Figure 2: Graphical representation of the relative expression of CD3D gene in blood and tumor tissue samplesFor comparative analysis the patients have been divided into groups: Group 1 Meningioma, Group 2 Medulloblastoma, Group 3 Ptiuitary adenoma and craniopharyngioma, Group 4- Glioma, Group 5 Glioblastoma and Group 6 Astrocytoma

Expression analysis of *KLRB1* gene:The *KLRB1* gene is expressed on lymphocytes found in mucosal tissues, as well as in blood, especially on Natural Killer T-cells, T helper 17 cells and a population of unconventional T cells known as Mucosal Associated Invariant T (MAIT) cellsshown in Figure 3(A1,A2 and B). The association of high CD161 expression with innate T cell populations including MAIT cells is established. Here we find that *KLRB1* (CD161) showed significant expression in the blood of patients with brain tumor. And also showed expression in the biopsy samples of histologically less-aggressive tumor meningioma, pituitary adenoma and in one glioblastoma (IDH mild type) patient**.**


Figure 3: Expression of *KLRB1* geneVisualization of 585bp product *KLRB1* gene on 1.2% agarose gel. **(A1)** Expression profile on blood samples (14-23) **(A2)** Expression profile on blood

samples (10, 10*-15). **(B)** Expression on tissue samples. Samples IDs: (**BA*** Blood Samples analysis**)** (**TA***Tissue Samples analysis **)**

The association of high CD161 expression with innate T cell populations including MAIT cells is established. Here we find that *KLRB1* (CD161) showed significant expression in the blood of patients with brain tumor. And also showed expression in the biopsy samples of histologically less-aggressive tumor meningioma, pituitary adenoma and in one glioblastoma (IDH mild type) patient**.**The graph represent the relative expression of *KLRB1* in blood and tissue samples, depicted that the expression of this gene is higher in the blood samples relative to the tissue sample except for meningioma, where the expression was higher in the tumor tissue compared to the blood as manifested on gel and also by graphical analysis as shown in Figure 4.In gliomas, both blood and tissue samples manifest lower expression of *KLRB1* gene.
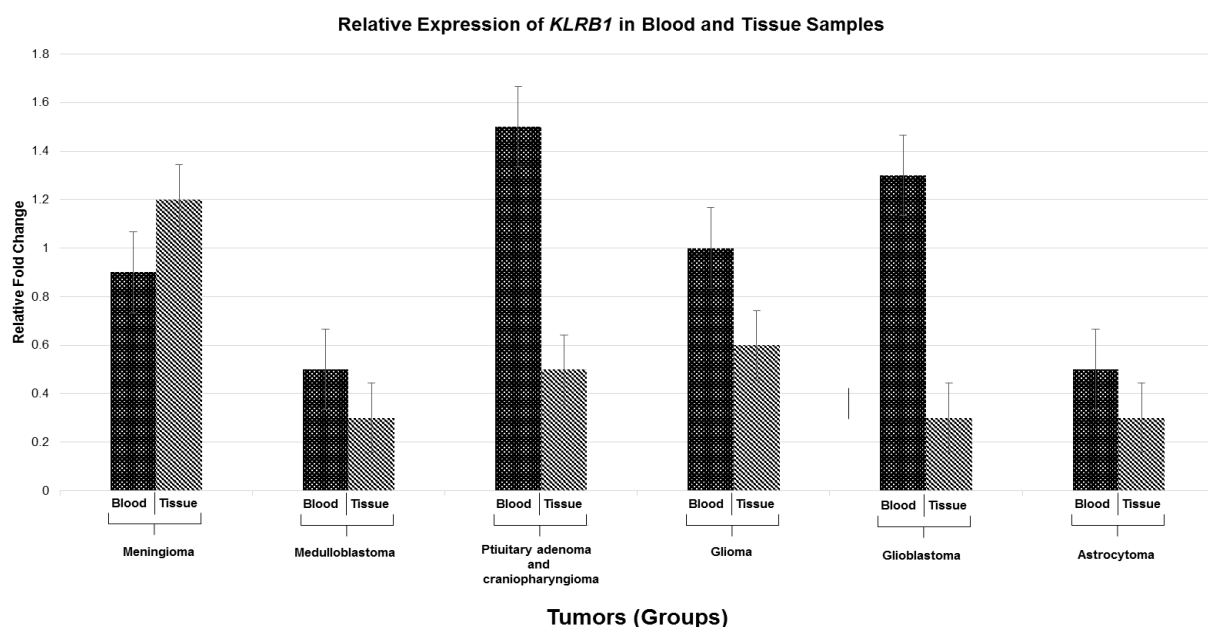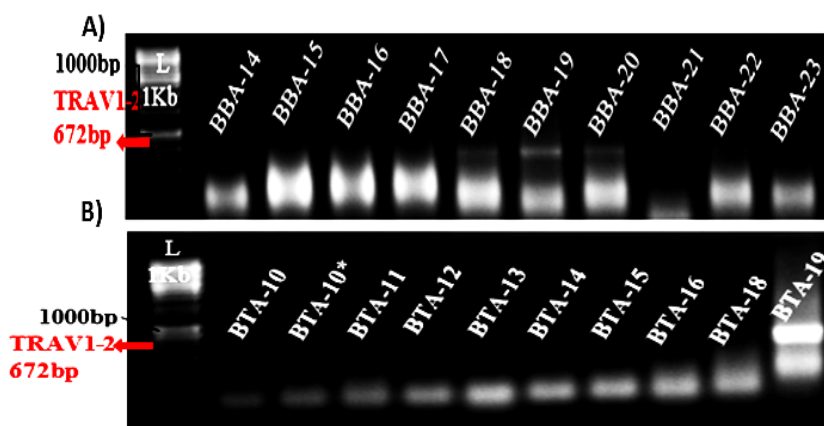

Figure 4: Graphical representation of the relative expression of *KLRB1* gene in blood and tumor tissue samples. For comparative analysis the patients have been divided into groups: Group 1 Meningioma, Group 2 Medulloblastoma, Group 3 Ptiuitary adenoma and craniopharyngioma, Group 4- Glioma, Group 5 Glioblastoma and Group 6 Astrocytoma

Expression analysis of *TRAV1-2* gene*:*
TRAV1-2 expression is specific to MAIT cells. On gel-electrophoresis this gene showed a very low expression in the blood samples of patients with meningioma, glioblastoma and pituitary adenoma. But showed no expression in the biopsy samples reflecting the absence of these cells in brain tumor setting. With the exception that *TRAV1-2* showed considerable expression in only a meningioma patient as shown in Figure 5(A and B).

Figure 5: Expression of *TRAV1-2* gene. Visualization of 672bp product CD3D gene on 1.2% agarose gel. (A) Expression profile on blood samples. (B) Expression on tissue samples.

From the graphical representation of data, we inferred that the *TRAV1-2*gene made little to no expression in the tumor tissue except for one meningioma tissue where the expression was significant. With relative to tumor tissue, this gene somehow showed a minor expression in the blood samples of patients with meningioma and pituitary adenoma patients **as shown in Figure 6**.
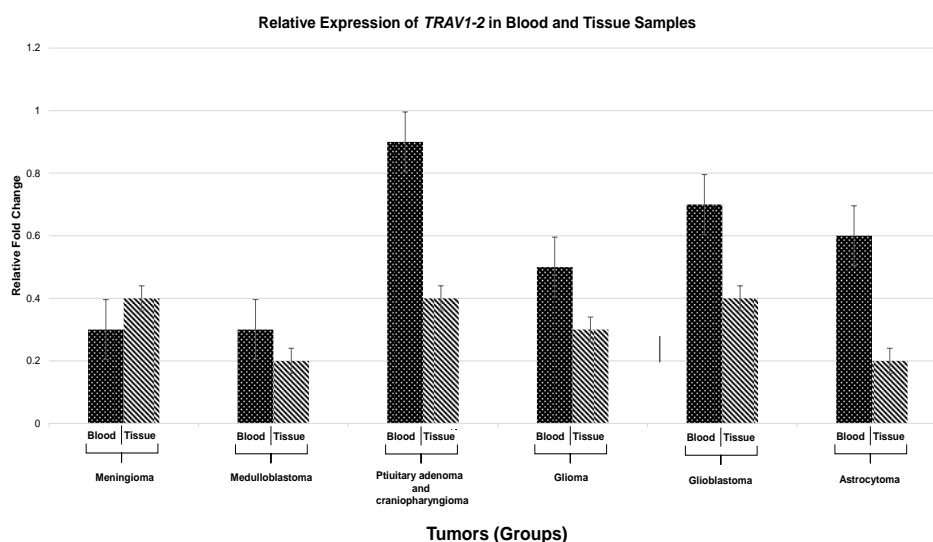
Figure 6: Graphical representation of the relative expression of *KLRB1* gene in blood and tumor tissue samples. For comparative analysis the patients have been divided into groups: Group 1 Meningioma, Group 2 Medulloblastoma, Group 3 Ptiuitary adenoma and craniopharyngioma, Group 4- Glioma, Group 5 Glioblastoma and Group 6 Astrocytoma

Disscussion

The MR1 molecule, contrary to the classical MHC class-I molecules, does not offer the peptide antigens to immune-system, preferably it offers the peptides (vitamin B metabolite of microbial origin) to an innate and un-conventional T-cell population known as MAIT cells[11]. These T cells are especially enriched in the peripheral blood, mucosal tissues and liver. MAIT cells possess an effector- memory characteristic and exhibit chemokine receptors and VLA-4 protein (very-late antigen 4 protein) on the surface of cells that aid in rolling of these cells across the BBB into the brain. This would be a mean for the MAIT cells to communicate with other brain resident cells including both immune and non-immune cells [12].

Since, MAIT cells' functional and phenotypic similarities to NKT cells have already been suggested, these cells are of particular interest for immunotherapists. The NKT cells and MAIT cells are the chief innate αβ T-lymphocyte subsets that infiltrate human tumors. It also reflects that the co-occurrence of theses innate T-cells subsets in human tumors is not coincidental but fairly specific. *KLRB1*, the CD161 encoding gene is the strongest positive prognostic marker in many solid tumors and is exhibit by many lymphocytes. The MAIT cells particularly express high levels of CD161 marker [13]. (Peterfalvi*et al.,* 2008). A dominant subset of polyclonal CD8^{+} T cells (including a population of CD8^{+} MAIT cells) showed high expression of *KLRB1* gene and also exhibit CD69 and CD103 markers, which are associated with the tissue residence of these cells. The expression of this gene is also associated with the elevated levels of cytotoxic mediators [14]. In agreement with MS lesion, the expression of CD161 (*KLRB1*) was found superior in the tumor tissue of Meningioma than in the peripheral blood suggesting the infiltration of these cells in the tumor lesion. However, the expression of *KLRB1* decreased as the malignancy of tumor increased i.e. the expression was found null in the glioma. The same pattern was observed in case of CD3 marker (*CD3D* gene)in other malignancies [15,16].

In our study the expression of MAIT TCRα-chain variant Vα7.2-Jα33 validated the presence of these cells in human tumors. Anticipation of anti-tumor properties of NKT cells have already begin in clinical trials to treat human cancers. The existence of invariant Vα24-JαQ TCR or NKT cells have been found in human tumor tissues including the tumor in situ. Though until recently the expression of Vα7.2-Jα33 (*TRAV1-2*) rearrangement was found and examined only in auto-immune diseases and sarcoid lesions. The restricted Vβ2 TCR and especially Vβ13 TCRs of MAIT cells have already been documented in a number of human tumors. Additionally, it has been reported the cytolytic potential of infiltrating Vβ13^{+} T cells, predominantly exhibiting CD8 phenotype, the characteristics possessed by MAIT cells [17]. Indeed, brain tumor as meningioma expressing the TCRα-chain variant Vα7.2 (*TRAV1-2*) of MAIT cells was infiltrated by CD8^{+} MAIT cells in our study. However, the glioma and other clinically advanced brain tumor subsets did no express the *TRAV1-2* suggesting absence of these cells in malignant tumors especially the glioma and glioblastoma [18].

Quite recently, a Bulk Tumor Transcriptomic study on MAIT cells occurrence in human cancers revealed that glioblastoma and low-grade gliomas, did not express the strong transcriptional signal of MAIT cell infiltration [19]. In immune-suppressive microenvironment,these cancerous cells secrets IL-10, VEGF, TGF-b, PDL-1, periostin, STAT3 etc. which inhibit the growth and proliferation of T- cells, diminishing pro-inflammatory signals, and facilitate the recruitment of TAMs-tumor-associated macrophages, MDSCs- myeloid derived suppressive cells and regulatory T cells (Tregs) at the site of tumor [20-21]. Therefore these unconventional T cells might be depleted (undergo apoptosis) in the highly suppressive brain TME or undergo activation induced cell death.

Certainly, lack of integrity of mucosal barriers ordinarily associated with immune-mediated and auto-immune diseases resulted in expanded exposure to microbial stimuli which resulted in prolonged activation and eventually exhaustion of MAIT cells [22, 23]. No expression of MAIT cell TCR a-chain variant (*TRAV1-2*) suggested the same exhaustion and depletion of these cells with increased clinical grade of tumor i.e. higher in meningioma tissue specimen to no expression in glioma and glioblastoma (emphasize the immunological differences of TME, located within the different brain compartments). However, further studies are required for detailed expression analysis in support of Tumor assessment methodologies.

Conclusion: The validation of the expression levels of these cells in human brain tumors and their contribution in brain tumor setting require further investigation. However, in the therapeutic setting, if the suppressive effects of the tumor microenvironment on MAIT cell activity can be abrogated by immune checkpoint inhibitors, these cells themselves could have considerable

therapeutic potential, as has been suggested for other innate T cells. Furthermore, MAIT cells naturally display high levels of the ABCB1 multi-drug efflux protein, which gives resistance to the harmful effects of chemotherapy. Contributing resistance to the deleterious effects of chemotherapy, which means that the contribution of MAIT cells to anti-tumor immunity may be especially prominent in the setting of combination therapy where chemo-sensitive CTLs are destroyed. Thus, MAIT cells might prove beneficial in immunotherapy against brain tumors as evident by references and results.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This study is approved by Institutional review board of Forman Christian College (A Chartered University) and informed consent of each patient was taken before enrollment.

HUMAN AND ANIMAL RIGHTS
No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

References
1. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014 Jul;16(7):896-913
2. Stone, Jennifer D, and Lawrence J Stern. "CD8 T cells, like CD4 T cells, are triggered by multivalent engagement of TCRs by MHC-peptide ligands but not by monovalent engagement." *Journal of immunology (Baltimore, Md. : 1950)* vol. 176,3 (2006): 1498-505.
3. Le Bourhis, Lionel et al. "Mucosal-associated invariant T cells: unconventional development and function." *Trends in immunology* vol. 32,5 (2011): 212-8.
4. Dias J, Boulouis C, Gorin JB, van den Biggelaar RHGA, Lal KG, Gibbs A, Loh L, Gulam MY, Sia WR, Bari S, Hwang WYK, Nixon DF, Nguyen S, Betts MR, Buggert M, Eller MA, Broliden K, Tjernlund A, Sandberg JK, Leeansyah E. The CD4⁻CD8⁻ MAIT cell subpopulation is a functionally distinct subset developmentally related to the main CD8⁺ MAIT cell pool. ProcNatlAcadSci U S A. 2018 Dec 4;115(49):E11513-E11522.
5. Xiao X, Cai J. Mucosal-Associated Invariant T Cells: New Insights into Antigen Recognition and Activation. Front Immunol. 2017 Nov 10;8:1540.
6. Nel, I., Bertrand, L., Toubal, A. *et al.* MAIT cells, guardians of skin and mucosa?. *Mucosal Immunol* 14, 803–814 (2021).

7. Bonaguro L, Schulte-Schrepping J, Netea MG, Händler K, Mukherjee S, Ulas T, Schultze JL, Aschenbrenner AC. Human variation in population-wide gene expression data predicts gene perturbation phenotype. iScience. 2022 Oct 12;25(11):105328.

8. Ussher JE, Klenerman P, Willberg CB. Mucosal-associated invariant T-cells: new players in anti-bacterial immunity. Front Immunol. 2014 Oct 8;5:450.

9. Sundström P, Szeponik L, Ahlmanner F, Quiding-Järbrink M. Tumor-infiltrating mucosal-associated invariant T (MAIT) cells retain expression of cytotoxic effector molecules. Oncotarget. 2019 Apr 19;10(29):2810-2823.

10. Mo J, Zheng L, Gao Z, Wu J, Bao Y. The Study of Mucosal-Associated Invariant T Cells in Colon Cancer and Roles in Immune Activities. Onco Targets Ther. 2021 Nov 16;14:5263-5273.

11. Chiba A, Murayama G, Miyake S. Mucosal-Associated Invariant T Cells in Autoimmune Diseases. Front Immunol. 2018 Jun 11;9:1333.

12. Trottein F, Paget C. Natural Killer T Cells and Mucosal-Associated Invariant T Cells in Lung Infections. Front Immunol. 2018 Aug 2;9:1750.

13. Cosgrove C, Ussher JE, Rauch A, Gärtner K, Kurioka A, Hühn MH, Adelmann K, Kang YH, Fergusson JR, Simmonds P, Goulder P, Hansen TH, Fox J, Günthard HF, Khanna N, Powrie F, Steel A, Gazzard B, Phillips RE, Frater J, Uhlig H, Klenerman P. Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. Blood. 2013 Feb 7;121(6):951-61.

14. Eberhard JM, Hartjen P, Schulze zurWiesch J. CD161+ MAIT cells are severely reduced in peripheral blood and lymph nodes of HIV-infected individuals independently of disease progression. PLoS One. 2014 Nov 4;9(11):e111323.

15. Fergusson JR, Hühn MH, Swadling L, et al. CD161(int)CD8+ T cells: a novel population of highly functional, memory CD8+ T cells enriched within the gut. Mucosal Immunol. 2016 Mar;9(2):401-13.

16. Shafique S, Haseeb HM, Bano K, Arshad A. Investigating Entosis and Autophagy Dynamics in Diverse Tumor Microenvironments , Pak-Euro Journal of Medical and Life Sciences: Vol. 7, 3 (2023) Dec: 219-226.

17. Di, Wang et al. "Clinical characterization and immunosuppressive regulation of CD161 (KLRB1) in glioma through 916 samples." *Cancer science* vol. 113,2 (2022): 756-769.

18. Peterfalvi, Agnes et al. "Invariant Valpha7.2-Jalpha33 TCR is expressed in human kidney and brain tumors indicating infiltration by mucosal-associated invariant T (MAIT) cells." *International immunology* vol. 20,12 (2008): 1517-25.

19. Shoaib M, Haseeb HM, Bano K, Arshad A. Unraveling the Complexity of Immune Checkpoint Modulation in Gastrointestinal Malignancies, Pak-Euro Journal of Medical and Life Sciences: Vol. 6, 3 (2023) Sep: 269-276.

20. Arshad A, Deutsch E, Vozenin MC. Simultaneous irradiation of fibroblasts and carcinoma cells repress the secretion of soluble factors able to stimulate carcinoma cell migration. PLoS One. 2015 Jan 30;10(1):e0115447.

21. Shafiq A, Moore J, Suleman A, Faiz S, Farooq O, Arshad A, Tehseen M, Zafar A, Ali SH, Din NU, Loya A. Elevated soluble galectin-3 as a marker of chemotherapy efficacy in breast cancer patients: A prospective study. International journal of breast cancer. 2020 Mar 14;2020.

22. Lukasik Z, Elewaut D, Venken K. MAIT Cells Come to the Rescue in Cancer Immunotherapy? Cancers (Basel). 2020 Feb 11;12(2):413.

23. Ahtzaz K, Ali M, Arshad A. Comparative analysis of exon 11 mutations of brca1 gene in regard to circulating tumor dna (ctdna) & genomic dna in a cohort of breast cancer patients in Pakistan, December 2017: Asian Journal of Science and Technology Vol. 08, Issue, 12, pp.7029-7046.