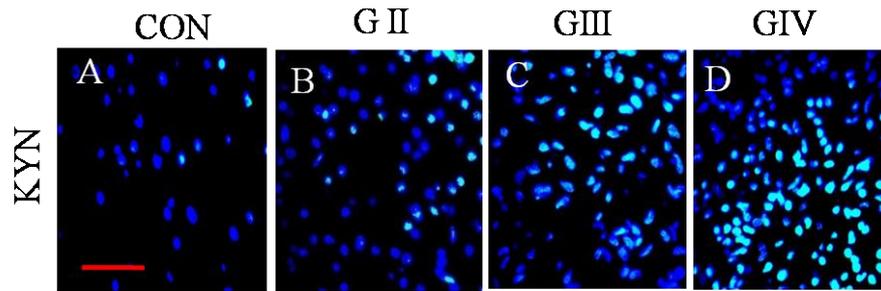


Supplementary File Method A Methodology of tissue processing and Immune-staining

Standard paraffin sections were used to check the status of isocitrate dehydrogenase (IDH)-1, p53, ATRX, Kynurenine (KYN), human telomerase reverse transcriptase (hTERT) and Olig2 using immunofluorescence based immunohistochemistry as *per* WHO guidelines 2016 (Louis *et al*, 2016). 30 glioma tissue sections in triplicates were compared with $n = 15$ non-malignant brain tissue samples as *per* lab protocol published earlier (Gandhi and Khare, Turk J Pathol, 2018). Primary monoclonal antibodies IDH-1 and KYN (Santa Cruz Biotechnology, United States, 1:1000 dilution), p53, ATRX and Olig2 (Bethyl Laboratories, United States, 1:1000 dilution) and compatible secondary anti-mouse and anti-rabbit (1:300 dilution, Santa Cruz Biotechnology, United States) were used. Using an epi-flourescence microscope (Carl Zeiss, AxioPlan2), an average of 10 hotspot areas expressing the marker of interest were considered and approximately 1000 cells *per* section were captured with 40-fold magnification. FITC signal was detected using Zeiss-09 filter set (excitation BP, 450/490 nm; beam splitter FT, 510; emission LP, 515 nm). The case-data manager expo-software by Applied Spectral Imaging (Israel) was used to analyze the images exported as TIFF files. Quantification of fluorescence signals emitted by the tagged molecular markers was carried out using Image-J software (National Institute of Health, United States).

In the IDH-*w* category, tissue was positive for EGFR and ATRX but had low expression of p53. The IDH-*m* group further presented two subtypes: 41.6% cases of oligodendro-glioma with OLIG2 stain, the rest 58.4% tissues were astrocytic gliomas as confirmed by p53 and ATRX immune-positivity.

Result: KYN and hTERT tissue based expression-Positive tissue based expression of KYN in paraffin sections was quantified followed by analysis using software Image-J. Expression in patient tissue was significant from controls, as also between IDH-*m/w* ($P < 0.0001$; Supplementary Figure 1). Correlation of tissue expression with corresponding plasma concentrations in circulation was found to be positively significant for KYN ($r = 0.5688$; $P = 0.001$).



Supplementary Figure 1 Immunofluorescence-immunohistochemistry based differential expression of Kynurenine in tissue sections A: Control showing negligible positivity; B: Minimal in grade II; C: Moderate in grade III; D: High expression in grade IV; Scale bar 50 μm ; E and F: The difference in Kynurenine expression in (E) isocitrate dehydrogenase (IDH)-*m* (F) IDH-*w*; is also evident. Scale bar: 100 μm . KYN: Kynurenine.

Supplementary Table 1 Receiver operating characteristic analysis of quantified expression of Kynurenine between different grades

| S.No. | Grades | AUC | STD. Error | 95%CI | Cut-off | Sensitivity (%) | P value |
|--------------|---------------------------|------|---------------|-----------|----------|--------------------|----------|
| KYN | | | | | | | |
| 1 | Con & G-II | 0.89 | 0.04 | 0.81-0.97 | > 16.18 | 80 | < 0.0001 |
| 2 | G-II & G-III | 0.69 | 0.06 | 0.55-0.83 | > 34.37 | 83.33 | 0.0095 |
| 3 | G-III & G-IV | 0.73 | 0.06 | 0.60-0.86 | > 90.03 | 83.33 | < 0.002 |
| 4 | Low & High | 0.78 | 0.04 | 0.68-0.87 | > 21.48 | 95 | < 0.0001 |
| 5 | IDH- <i>m</i> & <i>wt</i> | 0.79 | 0.05 | 0.69-0.89 | > 67.41 | 90 | < 0.0001 |
| hTERT | | | | | | | |
| 1 | Con & G-II | 0.68 | 0.07 | 0.53-0.83 | > 1.101 | 80 | 0.016 |
| 2 | G-II & G-III | 0.71 | 0.06 | 0.58-0.84 | > 1.319 | 73.33 | 0.0039 |
| 3 | G-III & G-IV | 0.51 | 0.07 | 0.36-0.66 | > 2.014 | 53.33 | 0.856 |
| 4 | Low & High | 0.71 | 0.05 | 0.60-0.81 | > 1.140 | 80 | 0.001 |
| 5 | IDH- <i>m</i> & <i>wt</i> | 0.76 | 0.04 | 0.71-0.81 | > 1.104 | 85 | 0.0001 |
| NLR | | | | | | | |
| 1 | Con & G-II | 0.81 | 0.06 | 0.69-0.92 | > 1.175 | 80 | < 0.0001 |
| 2 | G-II & G-III | 0.73 | 0.06 | 0.60-0.86 | > 3.035 | 80 | 0.0017 |
| 3 | G-III & G-IV | 0.62 | 0.07 | 0.48-0.76 | > 4.43 | 70 | 0.0963 |
| 4 | Low & High | 0.79 | 0.05 | 0.68-0.89 | > 2.54 | 95 | < 0.0001 |
| 5 | IDH- <i>m</i> & <i>wt</i> | 0.73 | 0.05 | 0.63-0.83 | > 3.95 | 80 | 0.0003 |
| IL-6 | | | | | | | |
| 1 | Con & G-II | 0.64 | 0.07 | 0.50-0.78 | > 41.98 | 80 | 0.04 |
| 2 | G-II & G-III | 0.65 | 0.07 | 0.51-0.79 | > 60.50 | 70 | 0.04 |
| 3 | G-III & G-IV | 0.82 | 0.05 | 0.72-0.93 | > 107.2 | 93.33 | < 0.0001 |
| 4 | Low & High | 0.81 | 0.04 | 0.73-0.90 | > 59.6 | 85 | < 0.0001 |
| 5 | IDH- <i>m</i> & <i>wt</i> | 0.95 | 0.02 | 0.89-1.0 | > 100.04 | 96.7 | < 0.0001 |

Receiver operating characteristic curve analysis of control (Con), grade II, grade III and grade IV provided an optimal cut-off value for Kynurenine. KYN: Kynurenine;

IL-6: Interleukin-6; NLR: Neutrophil lymphocytes ratio; hTERT: Human telomerase reverse transcriptase.