Immunological study on brain tumor patients

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Abstract
Current study aimed to accomplished by assessment levels of alkaline phosphatase and IL-12 levels in Iraqi patients with brain tumor. From November 2021 to February 2022, researchers at Al-Hussein Hospital in Karbala, Iraq, will investigate the prevalence of bacteremia and immunological markers in patients with brain tumors. 50 patients with brain tumors (27 males, 23 females) and 30 healthy people as control Both patients and controls had blood drawn. interleukin-12 (IL-12) by ELISA. The findings indicated that the most vulnerable age group was 1–10-year-old, with a 26% infection rate compared to other age groups. Interleukin-12 levels in patients are lower than in healthy controls
Keywords: Brain, IL-12, tumor

1. Introduction

As predicted, the human brain has the same amount of neurons as a monkey brain of comparable size (Marino *et al.* 2007). More than 80 percent of brain mass is ascribed to an overdeveloped cerebral cortex that contains 100 billion neurons and ten times as many glial cells. Countless neurons and non-neuronal cells were discovered therein (Herculano-Houzel 2009). When cells multiply and replicate uncontrollably in an intracranial tumor, commonly known as a brain tumor, they seem to be unchecked by the regular mechanisms that regulate cells. Primary and metastatic brain tumors are the two most frequent forms, however there are more than 150 other varieties. As a general rule, primary brain tumors grow from inside the brain's tissues or its immediate surroundings. Primary tumors in the brain, whether glial or non-glial (forming on or in the brain's nerves, blood vessels, or glands), are either benign or malignant. Metastatic brain tumors are cancers that originate elsewhere in the body (such as the breast or lungs) and then spread to the brain through the circulatory system. Cancerous tumors that have spread to other parts of the body are known as metastatic tumors (Wang *et al.* 2020).
There is immune factors that are affected by the tumor, including IL-12.
Malignant tumors in these patients are linked to the development and progression of interleukin (IL)-12, for example. IL-12 is considered a potential cancer immunotherapy approach due to its strong effect on the establishment of an anticancer immune response. IL-12 has been shown in multiple studies to successfully kill cancer cells both in vitro and in vivo. Tumor-associated macrophages' tumor-supportive activities may be drastically reduced by IL-12's antiangiogenic and antiangiogenic properties (Hong *et al.* 2022).
The study aims to study the immunological factors of brain tumor disease in Iraq .In this study, immunological changes occur in brain cancer patients were studied and compared with healthy patients. The study's aim is accomplished by Evaluation IL-12 levels.

2. Materials and Methods

Blood samples were collected from patients with brain tumor (50 samples and with 30 samples from healthy persons), where the blood sample was withdrawn from patients and healthy persons. Samples have been placed in a gel tubes and then centrifugation to obtained serum. The withdrawal of serum and its placement in the Pendroff tubes to be use in biochemical and immunological tests (IL-12) required according to procedure that provided with each kit.

3. Statistical Analysis

Chi-square tests were used in the analysis of data from SAS 2012. The results were compared using a less important difference (LSD) at a probability level of 0.05, 0.01.
The Data was analyzed in a CRD (Complete Randomized Design) In a practical way 2x2x6. The averages were compared with L.S.D (less important difference) and Chi -square at a probability level of 0.05, 0.01 using S A S 2012. SAS 2012. Statistical Analysis System, User,s Guide. Statistical. Version 9.1st ed. SAS. Institute Incorporated Cary. N.C. USA.

4. Results

The current study included 50 patients (27 male and 23 female) diagnosed by the physician with a brain tumor and these patients reviewed to the Warith International Cancer Institute in the city of Karbala, and 30 persons (12 male and 18 female) as control as shown in Table 4.1 and Table 4.2, which represents the number and proportion of patients according to the age group its observed in this study that the category of children aged 1-10 age was the most likely For injury, the rate of infection was 26% compared to the other age groups that were lower than this percentage. Also, through our study, which focused on the study of immune factors mentioned

doi.org/10.31838/hiv22.02.661 Received: 28.04.22, Revised:10.05.22, Accepted: 30.08.22

in the proposal , for IL-12, there is no significant difference between the healthy and the patients, but there is a mathematical difference where the level of IL-12 in patients is lower than in the healthy. and described in Table 4.4, Table 4.5 and Table 4.6.

Table 4.1 Demographic properties of studing groups			
Group properties	Number	Sex	percentage
Patients	50	M 27 F 23	%54 %46
Table 4.2 Age distribution of patients and control	Table 4.2 Age distribution of patients and control	Table 4.2 Age distribution of patients and control	Table 4.2 Age distribution of patients and control

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Age group	Number	percentage
10-1	13	%26
20-10	10	%20
30-20	7	%14
40-30	8	%16
50-40	6	%12
> 50	6	%12
Total	50	100

Table 4.4 Show IL-12 (pg/mL) levels in both groups	
Factor Group	IL-12
Patients	4.60 ±21.80
Controls	4.02 ±26.09
P value	n.s

5. Discussion

In our study and through the results obtained it was found that the level of interleukin-12 has decreased and this indicates that this cytokinase has an important role in reducing the spread of the tumor as in healthy people it was more than in patients and therefore it can be noted that this cytokinase has a role in the process of the formation and spread of tumor in the human body. It has emerged during the last two decades as one of the most powerful cytokines in preclinical models for promoting anticancer activity. Macrophages, T lymphocytes, and dendritic cells are all impacted by IL-12 in the tumor microenvironment (Tugues *et al.* 2015b). The cytokine IL-12 has the potential to greatly increase the anticancer immune response in preclinical studies. Immune responses to tumors caused by IL-12 are modulated by the kinds and locations of tumors, as well as the cytokines and effector cells involved. The delivery of IL-12 to the tumor site for therapeutic reasons necessitates new approaches. This cytokine is produced by APCs like dendritic cells (DCs), macrophages, and B cells when triggered by Toll-like receptors (TLRs). Research by Trinchieri and colleagues, 1993 Because of this, infections trigger the release of the proinflammatory cytokine IL-12. According to (Medzhitov 2001), Interferon-g (IFN-g), as well as other amplification signals, may be used (B. X. Ma et al., 1996) IFN-gamma (IL-10) and TGF-b1 (TGF-b1), on the other hand, inhibit the production of IL-12 (Du and Sriram 1998, Tugues *et al*. 2015b).

Cancer cells have been made to continually secrete IL-12 in order to understand how this cytokine causes antitumor immune responses. TSA breast adenocarcinoma and C26 colon carcinoma cells overexpressed with IL-12 underwent tumor suppression upon subcutaneous injection (Cavallo *et al.* 1997, Eisenring *et al.* 2010, Martinotti *et al.* 1993). When it came to the rejection of breast cancer TSA-IL-12 cells, IFN-g secreted by CD8 cytotoxic T cells was all that was needed, as opposed to ILCs in B16 melanoma (Cavallo *et al.* 1997) Tumor rejection in the central nervous system has been attributed in large part to T cells that are sensitive to IL-12 (Vetter *et al.* 2009, Berg *et al.* 2013). Tumor cell type and tumor location, as well as certain effector cell types and cytokines, have an impact on the quality of the immune response to tumors, according to these studies. IFNg-inducible chemokine levels are up and VEGF and metalloproteinase-9 synthesis are down when IL-12 is inhibiting tumor vasculature formation, according to research (Dias *et al.* 1998, Kanegane *et al.* 1998, Mitola *et al.* 2022).

Studies show that IL-12-induced IFN-g is an important tumor growth inhibitor (Tugues *et al.* 2015b, Cancer and York 2004). Even after tumor injection, IL-12's anticancer effects were still evident, and they were discovered to be partly reliant on CD8 T cells (Tugues *et al.* 2015b)

The supplied IL-12 was shown to activate myeloid cells and promote tumor antigen-specific CD8 T cells, which resulted in tumor regression (Kerkar *et al.* 2011, Lisiero *et al.* 2011, Zhang *et al.* 2009, Pegram *et al.* 2012). IL-12 delivered directly to the tumor site via immunocytokines is a relatively new development. Antibodies specific to the tumor's vascular system or necrotic core DNA have recently been fused with immunocytokines (Halin *et al.* 2002, Sommavilla *et al.* 2010), for example. Necrotic areas inside tumors are of special interest because of the

lack of circulation and cell death in solid tumors. Before utilizing antibody-targeted cytokines, a detailed evaluation of the tumor setting is necessary since significant avidity and retention to the targeted tissue are required for good therapeutic effects. Researchers found that targeting CD30 lymphoma cells with dual cytokine–antibody fusion proteins reduced tumor growth more efficiently than doing so with simply IL-12 or IL-2 as a single antigen in their treatment regimen.

IL-12 increases IFN production via activating CD8+ T cells and NK cells, which in turn increases IFN production. Studies have demonstrated that IFN- may kill tumor cells directly, stop the growth of blood vessels, increase the generation of NK cells, CTLs, and macrophages, all while increasing the expression of MHC I and II molecules on the tumor cell's surface. (Bromberg *et al.* 1996, Martini *et al.* 2010, Hayakawa *et al.* 2002, Rosa *et al.* 1986). Systemic injection of therapeutic IL-12, which has shown the ability to ameliorate local immune suppression in preclinical models, has been hampered by substantial inflammation-related adverse effects in clinical studies. To our knowledge, this is the first time that low doses of an antibody fusion protein (NHS–rmIL-12) have been successfully employed to inhibit syngeneic carcinomas with high immunologic activity (Hong *et al.* 2022). IL-12's anti-cancer properties have been repeatedly shown in preclinical carcinoma models (Noguchi *et al.* 1996, Zaharoff *et al.* 2010, Thomas *et al.* 2000). Clinical studies of Il-12 treating advanced cancer patients have been stymied by substantial adverse effects seen in early trials (Lacy *et al.* 2009, Higano *et al.* 1997, Hong *et al.* 2022). Cancer immunotherapy might benefit greatly from the addition of IL12. These cytokines are released by both immunological and malignant cells. The tumor cell cycle is blocked, apoptosis is induced, and tumor cell growth is prevented by treatments focused on the IL-12 family of cytokines. Therapies that target IL-12 family cytokines and immune checkpoint inhibition are synergistic in nature (Deplanque *et al.* 2017, Eckert *et al.* 2017, Mills *et al.* 2019, Guo *et al.* 2017).

6. Conclusions

The category of children with an age of 1-10 years is more likely in brain cancer from other age groups.

The Lack of significant difference in urea in brain cancer and healthy patients

For IL-12 , It has been found that his level has decreased in patients than in healthy .

References

Marino, L., Connor, R. C., Fordyce, R. E., Herman, L. M., Hof, P. R., Lefebvre, L., Lusseau, D., McCowan, B., Nimchinsky, E. A., Pack, A. A., Rendell, L., Reidenberg, J. S., Reiss, D., Uhen, M. D., Van Der Gucht, E. and Whitehead, H. 2007. Cetaceans have complex brains for complex cognition. PLoS Biology., 5(5): 0966–0972

Herculano-Houzel, S. 2009. The human brain in numbers: A linearly scaled-up primate brain. Frontiers in Human Neuroscience., 3: 1–11.

Hong, Y., Sievers, C., Allen, C. T., Hong, Y., Robbins, Y., Yang, X., Mydlarz, W. K., Sowers, A. and Allen, C. T. 2022. Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity., 7: 5.

Wang, C., Sinha, S., Jiang, X., Fitch, S., Wilson, C., Caretti, V., Ponnuswami, A., Monje, M., Grant, G. and Yang, F. 2020. A comparative study of brain tumor 44 cells from different age and anatomical locations using 3D biomimetic hydrogels. Acta Biomaterialia., 116: 201–208.

Tugues, S., Burkhard, S. H., Ohs, I., Vrohlings, M., Nussbaum, K., Vom Berg, J., Kulig, P. and Becher, B. 2015b. New insights into IL-12-mediated tumor suppression. Cell Death and Differentiation., 22(2): 237–246.

Medzhitov, R. 2001. Toll-like receptors and innate immunity. Nature Reviews Immunology., 1(2): 135-145.

Bromberg, J. F., Horvath, C. M., Wen, Z., Schreibert, R. D. and Darnell, J. E. 1996. Transcriptionally active Statl is required for the antiproliferative effects of both interferon a and interferon., 93: 7673–7678.

Du, C., and Sriram, S. 1998. Mechanism of inhibition of LPS-induced IL-12p40 production by IL-10 and TGF-β in ANA-1 cells. Journal of leukocyte biology., 64(1): 92-97.

Cavallo, F., Giovarelli, M., Forni, G., Signorelli, P., Musiani, P., Modesti, A., and Colombo, M. P. 1997. Antitumor efficacy of adenocarcinoma cells engineered to produce interleukin 12 (IL-12) or other cytokines compared with exogenous IL-12. Journal of the National Cancer Institute., 89(14): 1049-1058.

Martinotti, A., Stoppacciaro, A., Vagliani, M., Melani, C., Spreafico, F., Wysocka, M. and Colombo, M. P. 1995. CD4 T cells inhibit in vivo the CD8-mediated immune response against murine colon carcinoma cells transduced with interleukin-12 genes. European journal of immunology., 25(1): 137-146.

Vetter, M., Hofer, M. J., Roth, E., Pircher, H. P., and Pagenstecher, A. 2009. Intracerebral interleukin 12 induces glioma rejection in the brain predominantly by CD8+ T cells and independently of interferon-γ. Journal of Neuropathology & Experimental Neurology., 68(5): 525-534.

Berg, J., Vrohlings, M., Haller, S., Haimovici, A., Kulig, P., Sledzinska, A., Weller, M. and Becher, B. 2013. Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection. Journal of Experimental Medicine., 210(13): 2803–2811.

Dias, S., Boyd, R., and Balkwill, F. 1998. IL-12 regulates VEGF and MMPs in a murine breast cancer model. International journal of cancer., 78(3): 361-365.

Kanegane, C., Sgadari, C., Kanegane, H., Teruya-Feldstein, J., Yao, L., Gupta, G., and Tosato, G. 1998.

Contribution of the CXC chemokines IP-10 and Mig to the antitumor effects of IL-12. Journal of leukocyte biology., 64(3): 384-392.
Mitola, S., Strasly, M., Prato, M., Ghia, P., and Bussolino, F. 2022. IL-12 regulates an endothelial cell-lymphocyte network: effect on metalloproteinase-9 production. The Journal of Immunology., 171(7): 3725-3733.
Kerkar, S. P., Leonardi, A. J., Van Panhuys, N., Zhang, L., Yu, Z., Crompton, J. G., and Restifo, N. P. 2013. Collapse of the tumor stroma is triggered by IL-12 induction of Fas. Molecular Therapy., 21(7): 1369-1377.
Lisiero, D. N., Soto, H., Liau, L. M., and Prins, R. M. 2011. Enhanced sensitivity to IL-2 signaling regulates the clinical responsiveness of IL-12–primed CD8+ T cells in a melanoma model. The Journal of Immunology., 186(9): 5068-5077.
Zhang, L., Kerkar, S. P., Yu, Z., Zheng, Z., Yang, S., Restifo, N. P., Rosenberg, S. A. and Morgan, R. A. 2009. Improving Adoptive T Cell Therapy by Targeting and Controlling IL-12 Expression to the Tumor Environment. Molecular Therapy., 19(4): 751–759.
Pegram, H. J., Lee, J. C., Hayman, E. G., Imperato, G. H., Tedder, T. F., Sadelain, M. and Brentjens, R. J. 2012. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning., 119(18): 4133–4141.
Halin, C., Rondini, S., Nilsson, F., Berndt, A., Kosmehl, H., Zardi, L., and Neri, D. 2002. Enhancement of the antitumor activity of interleukin-12 by targeted delivery to neovasculature. Nature biotechnology., 20(3): 264-269.
Sommavilla, R., Pasche, N., Trachsel, E., Giovannoni, L., Roesli, C., Villa, A. and Neri, D. 2010. Expression , engineering and characterization of the tumor-targeting heterodimeric immunocytokine., 23(8): 653–661.
Martini, M., Grazia, M., Pasetto, M., Cristina, M., Innamorati, G., Mazzocco, M., Ugel, S., Cingarlini, S., Bronte, V., Zanovello, P., Krampera, M., Mosna, F., Cestari, T., Pia, A., Brutti, N., Barbieri, O., Matera, L., Tridente, G., Colombatti, M. and Sartoris, S. 2010. IFN- ⸗ -mediated upmodulation of MHC class I expression activates tumor-specific immune response in a mouse model of prostate cancer. Vaccine., 28(20): 3548–3557.
Hayakawa, Y., Takeda, K., Yagita, H., Smyth, M. J., Kaer, L. Van, Okumura, K. and Saiki, I. 2002. IFN- ⸗ –mediated inhibition of tumor angiogenesis by natural killer., 100(5): 1728–1733.
Rosa, F. M., Cochet, M. M. and Fellous, M. 1986. Interferon and major histocompatibility complex genes: a model to analyse eukaryotic gene regulation. Interferon., 7: 47-87.
Hong, Y., Sievers, C., Allen, C. T., Hong, Y., Robbins, Y., Yang, X., Mydlarz, W. K., Sowers, A. and Allen, C. T. 2022. Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity., 7: 5.
Noguchi, Y., Jungbluth, A., Richards, E. C. and Old, L. J. 1996. Effect of interleukin 12 on tumor induction by 3-methylcholanthrene. Proceedings of the National Academy of Sciences of the United States of America., 93(21): 11798–11801.
Zaharoff, D. A., Hance, K. W., Rogers, C. J., Schlom, J. and Greiner, J. W. 2010. Intratumoral immunotherapy of established solid tumors with chitosan/il-12. Journal of Immunotherapy., 33(7): 697–705.
Thomas, G. R., Chen, Z., Enamorado, I., Bancroft, C. and Van Waes, C. 2000. IL-12- and IL-2-induced tumor regression in a new murine model of oral squamous-cell carcinoma is promoted by expression of the CD80 co-stimulatory molecule and interferon-γ. International Journal of Cancer., 89(2): 368–374.
Lacy, M. Q., Jacobus, S., Blood, E. A., Kay, N. E., Rajkumar, S. V. and Greipp, P. R. 2009. Phase II study of interleukin-12 for treatment of plateau phase multiple myeloma (E1A96): A trial of the Eastern Cooperative Oncology Group. Leukemia Research., 33(11): 1485–1489.
Higano, B. C. S., Chielens, D., Raskind, W., Bryant, E., Flowers, M. E. D., Radich, J., Clift, R. and Appelbaum, F. 1997. 1997 by The American Society of Hematology,. 12, 9–14.
Hong, Y., Sievers, C., Allen, C. T., Hong, Y., Robbins, Y., Yang, X., Mydlarz, W. K., Sowers, A. and Allen, C. T. 2022. Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity Cure of syngeneic carcinomas with targeted IL-12 through obligate reprogramming of lymphoid and myeloid immunity., 7: 5.
Deplanque, G., Shabafrouz, K. and Obeid, M. 2017. Can local radiotherapy and IL-12 synergise to overcome the immunosuppressive tumor microenvironment and allow "in situ tumor vaccination" Cancer Immunology, Immunotherapy., 66(7): 833–840.
Eckert, F., Jelas, I., Oehme, M., Huber, S. M., Sonntag, K., Welker, C., Gillies, S. D., Strittmatter, W., Zips, D., Handgretinger, R. and Schilbach, K. 2017. Tumor-targeted IL-12 combined with local irradiation leads to systemic tumor control via abscopal effects in vivo. OncoImmunology., 6: 6.
Mills, B. N., Connolly, K. A., Ye, J., Murphy, J. D., Uccello, T. P., Han, B. J., Zhao, T., Drage, M. G., Murthy, A., Qiu, H., Patel, A., Figueroa, N. M., Johnston, C. J., 40 Prieto, P. A., Egilmez, N. K., Belt, B. A., Lord, E. M., Linehan, D. C. and Gerber, S. A. 2019. Stereotactic Body Radiation and Interleukin-12 Combination Therapy Eradicates Pancreatic Tumors by Repolarizing the Immune Microenvironment. Cell Reports., 29(2): 406-421.e5.
Guo, N., Wang, W. Q., Gong, X. J., Gao, L., Yang, L. R., Yu, W. N. and Zhao, Y. 2017. Study of recombinant human interleukin-12 for treatment of complications after radiotherapy for tumor patients. World journal of clinical oncology., 8(2): 158.
Bromberg, J. F., Horvath, C. M., Wen, Z., Schreibert, R. D. and Darnell, J. E. 1996. Transcriptionally active Statl is required for the antiproliferative effects of both interferon a and interferon., 93: 7673–7678.