

Expression of Death Receptor 3 (DR3) on Circulating CD4 Positive Lymphocytes in Patients with Alopecia Areata

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Abstract

Alopecia areata (AA) is an autoimmune disorder characterized by transient hair loss and preservation of the hair follicle that affect both sexes. There is scarcity in the published data that discuss the role of DR3 in pathogenesis and prognosis of AA. Therefore, our study was conducted to study this role by assessment of DR3 expression on circulating CD4 positive lymphocytes in AA patients. This study was a case control study that included 50 patients with AA in addition to 50 apparently healthy individuals of matched age and sex as a control group. Assessment of DR3 expression on circulating CD4 positive lymphocytes on both groups was done by flow cytometric analysis. Results of the present study demonstrated a significant difference in DR3 expression level between AA patients and control group. DR3 expression level was considered as risk predictor of AA occurrence. In addition, it was considered as risk predictor of active and severe (higher SALT score) cases.

1. Introduction

Alopecia areata (AA) is an immune system issue portrayed by transient, non-scarring going bald and safeguarding of the hair follicle. Balding can take numerous structures going from misfortune in all around characterized patches to diffuse or add up to going bald, which can influence all hair bearing locales [1].

Numerous hypotheses have been created with respect to the etiology of AA. At first, the contribution of an irresistible or poisonous specialist was viewed as causative, at that point neuropathic and endocrine unsettling influence speculations were introduced. At long last, the autoimmunity theory picked up ubiquity. The idea of autoimmunity in AA is bolstered by the high pace of relationship with other resistant interceded infections and the reaction seen to safe altering medicines [2].

Studies on morphology of hair follicles in AA have demonstrated 3 unmistakable examples of cell degeneration in factor frequencies, including dull cell change, apoptosis and putrefaction. Necroptosis additionally can possibly be related with the pathogenesis of AA. Necroptosis shows morphological highlights like rot. In contrast to rot, cell demise in necroptosis is profoundly managed by a modified intercellular stage, as in apoptosis. Tumor putrefaction factor (TNF)- α is the inducer of necroptosis and considered a significant cytokine associated with the pathogenesis of AA [3].

Passing receptors 3 (DR3) have a place with TNF receptor gene superfamily, which is characterized by comparable, cysteine-rich extracellular area. The passing receptors contain what's more a homologous cytoplasmic succession named the "demise space". Demise spaces commonly empower passing receptors to draw in the cell's apoptotic hardware, yet in certain examples they intercede capacities that are unmistakable from or even balance apoptosis. A few particles that communicate signals from death receptors contain demise spaces themselves [4].

Passing receptor 3, known as tumor putrefaction factor receptor superfamily part 25 (TNFRSF 25), is a phone surface receptor of the tumor rot factor receptor superfamily which intercedes apoptotic flagging and separation [5]. Its solitary known TNFSF ligand is TNF-like protein 1 A (TL1A) [6].

The association of TL1A and its receptor DR3 has been seen as engaged with the pathogenesis of a few provocative and immune system maladies, for example, rheumatoid joint pain, fiery gut sickness, psoriasis and numerous sclerosis [7].

Tumor rot factor receptor superfamily part 25 is for the most part communicated on T-lymphocytes and is up directed quickly by grafting including protein kinase c following their enactment by T cell receptor (TCR) incitement. The underlying capacity portrayed for TNFR25 on T-cell improved human lymphocytes was to upgrade T cell responsiveness to IL2 [8].

2. Subjects and methods

This investigation was a case-control study. This investigation included 50 patients with AA. Notwithstanding 50 evidently solid people of coordinated age and sex as a benchmark group. All patients were enrolled from the outpatient center of Dermatology, Venereology and Andrology division of Benha University Hospitals in the period from August 2019 to December 2019.

The investigation was affirmed by the morals advisory group of examination including human subjects of Benha Faculty of Medicine. Educated assent was acquired from every person before being taken a crack at the investigation

The examination included patients experiencing distinctive clinical sorts and degrees of seriousness of AA who were analyzed clinically and the analysis was affirmed by dermoscopy. The period of patients extended (18-45) years and both genders were included.

Coming up next were avoided from the investigation: Patients with other immune system sicknesses e.g: rheumatoid joint pain, incendiary entrail

illness and fundamental lupus erythematosus, Patients with different reasons for hair falling eg: telogen exhaust and cicatricial alopecia rejected by clinical assessment and Patients with malignant growth colon, disease prostate and disease bosom.

Appraisal of DR3 articulation on coursing CD4 positive lymphocytes on the two gatherings was finished by stream cytometric investigation.

Stream cytometry is a strategy used to distinguish and quantify physical and compound qualities of a populace of cells or particles. In this procedure, an example is suspended in a liquid and infused into the stream cytometer instrument. The example is engaged to in a perfect world stream each cell in turn through a laser bar, where the light dispersed is trademark to the cells and their parts. Cells are named with fluorescent markers so light is consumed and afterward radiated in a band of frequencies. A huge number of cells can be immediately inspected and the information accumulated are handled by a PC [9].

Stream cytometry utilized in biomarker discovery, cell tallying, cell arranging, deciding cell qualities and capacity, identifying microorganisms and protein designing recognition [10].

3. Results

The AA patients mean age was 29 years, they were 33 males (66%) and 17 females (34%). In addition to 50 healthy control group, their mean age was 30.9 years, they were 32 males (64%) and 18 females (36%). Patients and control groups had matched age and gender ($p>0.05$ for each).

No significant difference was found regarding family history between patients and control groups (16% versus 6%, $p>0.05$).

Assay of death Receptor 3 (DR3) expression on circulating CD4 positive lymphocytes were done. AA group showed significantly higher DR3 when compared to control group (mean=25.3% versus 15.9%, $p<0.001$).

Table (1) Comparison between AA patients and control group regarding DR3 level.

| | Control group N=50 | | AA patients N=50 | | Test | P |
|---------|--------------------|-----|------------------|-----|---------|--------|
| | mean | ±SD | mean | ±SD | | |
| DR3 (%) | 15.9 | 5.3 | 25.3 | 8.1 | T=4.641 | <0.001 |

SD, standard deviation; student t test was used for numerical parameters.

Receiver operating characteristic (ROC) curve of DR3 expression level was conducted for discrimination between AA patients and control group. DR3 showed

fair AUC (AUC=0.750). At cut off value of 15.7%, sensitivity was 76%, specificity was 64%, PPV was 67.9%, NPV was 72.7%, and accuracy was 70%.

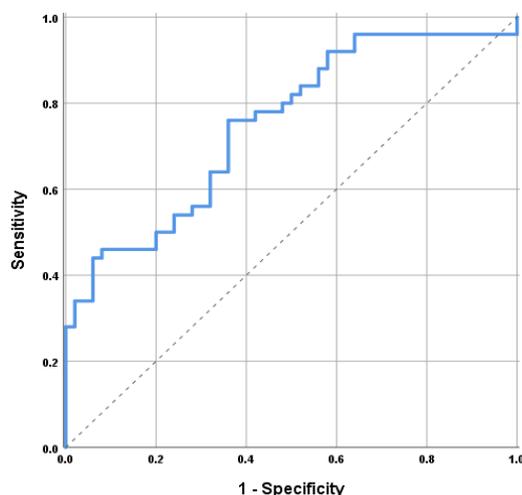


Fig (1) ROC curve of DR3 level for discrimination between AA patients and control group.

4. Discussion

As indicated by the most recent data we know, this is the principal study that examined the job of DR3 in pathogenesis, movement and guess of AA and its likelihood to be demonstrative biomarker. Hence, the current investigation was done to examine this job by

appraisal of DR3 articulation on flowing CD4 positive lymphocytes in patients with AA.

Consequences of this investigation demonstrated that DR3 articulation level was altogether higher in AA situations when contrasted with control gathering, just as it was fundamentally corresponded with SALT score and action. DR3 articulation level was considered as

hazard indicator of AA event. What's more, it was considered as hazard indicator of dynamic cases and higher SALT score.

Consequences of the current investigation can be clarified by the accompanying speculations. Significant advancement in fundamental and clinical immunology research recommends that AA is a CD8+ cell, Th1-type immune system response against anagen stage hair follicles. Numerous other cell types, including keratinocytes, fibroblasts, pole cells and dendritic cells likewise add to AA pathogenesis, planning with T cells. Safe movement is especially enhanced in the skin and discrete populaces of inhabitant and recycling memory T cells with contrasting domains of relocation and unmistakable useful exercises have been recognized. Along these lines it was set up that the guideline components by which lymphocytes, especially CD8+ cytotoxic T lymphocytes (CTLs), may add to AA pathogenesis and propose potential methodologies that lymphocytes can be balanced to control the improvement of this basic immune system ailment [11].

Another proof can be deciphered by examines indicated that TL1A and its receptor DR3 apply pleiotropic impacts on cell expansion, initiation, and separation of invulnerable cells, including assistant T cells and administrative T cells. TL1A and its receptors DR3 articulation is expanded in both serum and excited tissues in immune system maladies, for example, incendiary inside ailment (IBD), rheumatoid joint pain (RA), and ankylosing spondylitis (AS). Polymorphisms of the TNFSF15 quality that encodes TL1A are related with the pathogenesis of bad tempered entrail condition, uncleanliness, and immune system ailments, including IBD, AS, and essential biliary cirrhosis (PBC). In mice, obstructing of TL1A-DR3 cooperation by either opposing antibodies or erasure of the DR3 quality lessens the seriousness of numerous immune system sicknesses, while supported TL1A articulation on T cells or dendritic cells initiates IL-13-subordinate little intestinal aggravation. This recommends regulation of TL1A-DR3 cooperation might be an expected helpful objective in a few immune system maladies, including IBD, RA, AS, and PBC [12].

Total proof from examines proposes the commitment of DR3 in numerous resistant procedures and in different immune system ailments sharing pathogenic pathways like AA. For instance, DR3 was essentially communicated on fringe blood mononuclear cells of patients with psoriasis vulgaris [7]. Different investigates show the job of TL1A-DR3 in the pathogenesis of RA [13]. This could help the job of DR3 in pathogenesis of AA too.

The current investigation results uncovered expanded DR3 articulation level in patients with dynamic AA in contrast with tolerant with in-dynamic AA. This finding is bolstered by the finding that IL-17 level is higher in patients with dynamic AA (14), and that DR3 is firmly connected to IL17 in RA (13) and in IBD (15). T assistant sort 17 (Th17) cells play a

significant pathogenic capacity in immune system sicknesses; their guideline, be that as it may, isn't surely known. Studies indicated that the articulation DR3 is specifically raised in Th17 cells, and that TL1A, its related ligand, can advance the expansion of effector Th17 cells. Complete DR3 articulation is expanded in Th17 cells as contrasted and Th1 and Th2 cells, and full-length transmembrane DR3 articulation is expanded in Th17 cells as contrasted and Treg cells. Therefore, TL1A-DR3 collaboration may specially follow up on Th17 cells and differentially influence the separation and development of Th17 cells. Then again, TL1A restrains Th17 cell separation even within the sight of hostile to IL-2 killing immune response in vitro. Initiation of STAT1 flagging is instigated by fiery cytokines, for example, IL-27, IFN- γ , and type I IFN and restrains Th17 separation in vitro [16].

Demise receptor 3 (DR3) indicated huge positive connection with malady seriousness as per SALT score in AA patients. These outcomes were helped by the discoveries that degrees of TNF- α were higher in patients with greater expansions, higher evaluations of AA and with broad structures; totalis and universalis contrasted with different examples of AA (14), And upheld by the discoveries that DR3 level is corresponded with expanded degrees of TNF- α in RA patients. DR3-TL1A incites T cells to emit TNF- α and IL-17 under TCR incitement or Th17 polarization conditions, individually and it synergizes with IFN- γ and expands the creation of CXCL8 and grid metalloproteinase 9 in the human monocytic cell line THP-1. These provocative cytokines and chemokines are related with RA pathology, and hence it is conceivable that TL1A and these incendiary cytokines structure a horrible circle that bothers RA pathogenesis. For sure, organization of the counter TNF- α monoclonal immunizer adalimumab diminishes serum TL1A levels in RA patients [17].

References

- [1] K.J.MacLean and Tidman ,Alopecia areata: more than skin deep.The practioner, Vol.257, PP.29-32, 2013.
- [2] F.Rajabi, L.A.Drake, M.M.Senna and N.Rezaei , Alopecia areata: a review of disease pathogenesis. British J Dermatol, Vol. 179(5), PP.1033–1048, 2018.
- [3] Y.H.Jang, M.Jin, S.Y.Moon, W.J.Lee, S.J.Lee, M.K.Kim , Alopecia Areata : Investigation on the role of necroptosis .J AM Acad Dermatol, Vol.75(2), PP.436-439, 2016.
- [4] A.Ashkenazi and V.M.Dixit , Death Receptors: Signaling and Modulation.Science, Vol.281, pp.1305-1307,1998.
- [5] J.Kitson, T.Raven, Y.P.Jiang, D.V.Goeddel, K.M.Giles, K.T.Pun , A death-domain-containing receptors that mediate apoptosis. Nature, Vol.384,372-5, PP.1996.

- [6] E.C.Wang , On death receptor 3 and its ligands. *Immunology*; vol.137(1), PP.114-6, 2012.
- [7] L.Li, Y.Lu, L.Fu, Expression of DR3 on peripheral blood mononuclear cells of patients with psoriasis vulgaris. *Postgrad Med J*, Vol.94, PP.551-555, 2018.
- [8] T.H.Schreiber, D.Wolf and E.R.Podak, The Role of TNFRSF25:TNFSF15 in Disease and Health. *AdvExp Med Biol*, Vol.691, PP.289-298, 2011.
- [9] J.Picot, C.L.Guerin, C.Le Van Kim and C.M.Boulanger , "Flow cytometry: retrospective, fundamentals and recent instrumentation". *Cytotechnology*, Vol.64 (2), PP.109–30, 2012.
- [10] L. Givan and Alice, *Flow Cytometry Protocols. Methods in Molecular Biology*. Humana Press, Vol.36, PP.1–29, 2011.
- [11] R.Watanabe, A.Gehad, C.Yang, L.L.Scott, J.E.Teague, C.Schlapbach ,): Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. *Sci Transl Med*, Vol.(7), PP. 279-39, 2015.
- [12] Y.Aiba and M.Nakamura, The Role of TL1A and DR3 in Autoimmune and Inflammatory Diseases. *Mediators of Inflammation*, Vol.2013, PP.1–9, 2013.
- [13] G.Bamias, K.Stamatelopoulos, E.Zampeli, Circulating levels of TNF-like cytokine 1A correlate with the progression of atheromatous lesions in patients with rheumatoid arthritis. *Clini Immunol*, Vol.147(2), PP.144–150, 2013.
- [14] M.K.Tembhre and V.K.Sharma , T-helper and regulatory T-cell cytokines in the peripheral blood of patients with active alopecia areata. *Br J Dermatol*, Vol.169(3), pp . 543-548, 2013.
- [15] G.Bamias, G.Kaltsa, S.I.Siakavellas, High intestinal and systemic levels of decoy receptor 3 (DcR3) and its ligand TL1A in active ulcerative colitis. *Clini Immunol*, Vol.137(2), PP.242–249, 2010.
- [16] G.W.Jones, J.S. Stumhofer, T. Foster, Naive and activated T cells display differential responsiveness to TL1A that affects Th17 generation, maintenance, and proliferation. *Feder Ameri Soci J*, Vol.25(1), PP.409–419, 2011.
- [17] A.Hashiramoto, Y.Konishi, K.Murayama, H.Kawasaki, K.Yoshida, K.Tsumiyama , A variant of death-receptor 3 associated with rheumatoid arthritis interferes with apoptosis-induction of T cell . *J Biol Chem*, Vol.293(6), PP.1933–1943, 2018.