Cytokines play an important role in anti-mycobacterial response and may determine the type of tuberculosis. Thus, gene polymorphisms associated with cytokine production may be associated with susceptibility to TB. The objective of our study was to analyze the frequency of cytokine gene polymorphism (INF-γ [UTR]5644) in tuberculosis patients. Methods: This case-control study performed on 100 patients with tuberculosis and 194 healthy blood donors. DNA of blood sample of the people was purified by DNA extraction kit, then cytokine gene polymorphism interferon-g [A/T untranslated region (UTR)5644] was determined by cytokine gene polymorphism SSP kit (Heidelberg University, Heidelberg, Germany) and PCR-SSP method. The product obtained of PCR on agarose was electrophoresed. Gene frequencies were higher in PTB than control (P = 0.016). There was no difference in EPTB and control groups (P = 0.48). Frequencies of high producing INF-γ (A/T) were significantly over-represented in PTB group in comparison to both control and EPTB groups. Conclusion: Our results suggest that INF-γ high-producer polymorphism is associated with PTB. INF-γ genes polymorphism play key roles in susceptibility to or protection against tuberculosis development in the Iranian population.