Nickel Oxide (NiO) is a p-type metal oxide semiconductor which is widely used in many electronic and optoelectronic applications. The NiO use for such applications requires its optimum physical properties for the better performance of the fabricated devices. In this work, NiO thin film was deposited on a glass substrate by radiofrequency (RF) magnetron sputtering system using NiO sputtering target in argon plasma. The substrate temperature was increased from room temperature (RT) to 150 °C and then to 350 °C to study its effect on structural, morphological, optical and electrical properties of the film. Structural characterization of the NiO was made using x-ray diffraction (XRD) which showed an intense NiO (200) peak at RT, indicating its cubic crystal structure. By increasing the substrate temperature up to 350 °C, the crystallinity of the NiO was decreased. The decrease in the film's crystallinity was attributed to a partial thermal dissociation of the NiO at the glass substrate. Surface morphology of NiO on glass was studied using Field emission Scanning Electron Microscope. The results of surface morphology indicated a decrease in the size of grains and inhomogeneous granular structure with the increase of substrate temperature. The UV-Vis transmittance spectra of the NiO films were obtained to find out the film's band gap at different substrate temperatures. The results revealed a decrease in the band gap of the film by increasing the substrate temperature from RT to 350 °C which was associated with the defects induced energy levels within the band gap of NiO at the higher substrate temperature. Similarly, the electrical resistivity values of the NiO deposited on glass at different substrate temperatures were obtained using Four Probe method. The resistivity decreased due to increase in the substrate temperature which was ascribed to the changes in the film's crsytallinity.